

**MANIFESTATIONS
AND PATHOGENESIS OF CHLAMYDIAL
PELVIC INFLAMMATORY
DISEASE**

by

Pål Wølner-Hanssen

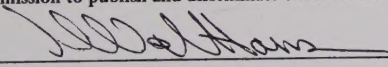
Lund 1985



Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION	
	Date of issue 1985-06-14	
	CODEN:	
Author(s) Pål Wølner-Hanssen	Sponsoring organization	
Title and subtitle Manifestations and pathogenesis of chlamydial pelvic inflammatory disease		
Abstract Between January 1975 and March 1983, 1031 women underwent laparoscopy for presumed pelvic inflammatory disease (PID); 656 (63.6%) had salpingitis. Clinical signs and symptoms among 104 women with presumed PID and chlamydial infection, were compared between women who did or did not have salpingitis. Elevated erythrocyte sedimentation rate and metrorrhagia had the highest predictive values for salpingitis (87% and 88%, respectively). In a subset of 54 women with presumed PID endometrial cultures for <u>Chlamydia trachomatis</u> were performed to help define the route of spread of the microorganism in the genital tract. Endometrial aspirates were positive for <u>C. trachomatis</u> in 12 of 26 women with and in 3 of 28 women without salpingitis ($p = 0.004$); in 6 of 8 with and in 2 of 9 without endometritis ($p = 0.04$). In women with chlamydia associated salpingitis, spread to non genital sites was also suggested. Seven patients had chlamydia associated salpingitis and periappendicitis. Spread of the inflammation from the right tube to the appendix seems probable. Seventeen patients had salpingitis and perihepatitis: all were infected with <u>C. trachomatis</u> , but not with <u>Neisseria gonorrhoeae</u> . Cultures from the liver surface of 2 of these patients yielded <u>C. trachomatis</u> , suggesting microbial spread from the tubes to the liver. Oral contraceptive users, presenting with presumed PID, were found to have laparoscopic signs of salpingitis significantly less often than users of barrier methods/no method ($RR = 0.27$). A negative correlation was found between oral contraceptive (OC) usage and complication of chlamydial salpingitis with perihepatitis (relative risk of OC usage versus barrier/no method = 0.07). This suggests that OC usage can modify the natural history of PID. Second-look laparoscopy after treatment of acute salpingitis in 13 women showed that fresh peritubal adhesions may resolve spontaneously. Attachment of <u>C. trachomatis</u> to human spermatozoa was studied in vitro. Increasing acidity, incubation time and concentration of inclusion forming units each independently enhanced such attachment. Second trimester and term amniotic fluids were found to possess chlamydiostatic properties in a test system using McCoy cell cultures.		
Key words Amniotic fluid, <u>Chlamydia trachomatis</u> , chlamydial attachment, endometritis, endometrial aspiration, Fitz-Hugh-Curtis syndrome, laparoscopy, oral contraceptives, pelvic inflammatory disease, perihepatitis, periappendicitis, salpingitis, spermatozoa		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title		ISBN
Recipient's notes	Number of pages	Price
	Security classification	

Distribution by (name and address) Pål Wølner-Hanssen, Department of Obstetrics and Gynecology
University Hospital, S-221 85 LUND Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature 

Date 85-03-26

MANIFESTATIONS
AND PATHOGENESIS OF CHLAMYDIAL
PELVIC INFLAMMATORY
DISEASE

av

Pål Wölner-Hansen
med. lic., Ld.

Akademisk avhandling som med vederbörligt
tillstånd av Medicinska Fakulteten vid
Lunds Universitet för avläggande av medi-
cine doktorsexamen kommer att offentligen
försvaras i kvinnokliniken i Lund före-
onsdagen den 4 sept.
läsningssal ~~fredagen den 14 juni~~ 1985,
kl 1315.

MANIFESTATIONS
AND PATHOGENESIS OF CHLAMYDIAL
PELVIC INFLAMMATORY
DISEASE

by

Pål Wølner-Hanssen

Department of Obstetrics and Gynecology and

Department of Medical Microbiology

University of Lund

Lund, Sweden

Phototype setting 1985 by Acta Obstetricia et Gynecologica Scandinavica

Box 443, S-901 09 Umeå, Sweden

ISBN 91-7626-057-7

Printing and binding by
Nyheternas Tryckeri AB, Umeå, Sweden, 1985

CONTENTS

ORIGINAL PAPERS	5
ABBREVIATIONS	6
INTRODUCTION	7
Chlamydia	7
Genital chlamydial infections in the female	7
Route of spread of genital pathogens in the female	8
Clinical and laparoscopic findings in patients with pelvic inflammatory disease	9
Evaluation of adnexa after an episode of pelvic inflammatory disease	9
Factors with possible impact on the spread of microorganisms from the lower genital tract	10
AIMS OF THE INVESTIGATION	11
MATERIALS AND METHODS	12
Patients	12
Sperm donors	12
Amniotic fluid donors	12
Laparoscopy	12
Sampling of culture specimens	12
Cervix	12
Urethra/Rectum	12
Endometrium	13
Fallopian tubes	13
Liver surface	13
Sampling sticks and transport media	13
Chlamydial culture	13
Chlamydial growth in presence of amniotic fluid	13
Gonococcal culture	14
Mycoplasma culture	14
Serology	14
Purification and incubation of chlamydia and spermatozoa	14
Detection of chlamydial attachment to spermatozoa	15
Immunofluorescence technique	15
Electron microscopy	15
Sperm penetration test	15
Statistical analysis	15

RESULTS AND COMMENTS	16
Route of spread of <i>C. trachomatis</i> from the cervix (I – IV)	16
Endometritis	16
Periappendicitis	17
Perihepatitis	17
Clinical vs laparoscopic findings in patients with chlamydial infection (V)	20
Laparoscopic findings	20
Clinical findings	20
Second look laparoscopy (VI)	21
Factors with possible impact on bacterial spread from the lower genital tract (VII – IX)	22
Contraception	22
Spermatozoa	23
Amniotic fluid	23
SUMMARY	24
ACKNOWLEDGEMENTS	25
REFERENCES	26
APPENDIX: PUBLICATIONS I – IX	

ORIGINAL PAPERS

This dissertation is based on the following papers:

- I. Wølner-Hanssen P, Mårdh P-A, Møller BR, Weström L. Endometrial infection in women with chlamydial salpingitis. *Sex Transm Dis* 1982;9:84–8.
- II. Mårdh P-A, Wølner-Hanssen P. Periappendicitis and chlamydial salpingitis. *Surg, Gynecol & Obstet* 1985; 160:304–6.
- III. Wølner-Hanssen P, Weström L, Mårdh P-A. Perihepatitis and chlamydial salpingitis. *Lancet* 1980;1:901–4.
- IV. Wølner-Hanssen P, Svensson L, Weström L, Mårdh P-A. Isolation of *Chlamydia trachomatis* from the liver capsule in Fitz-Hugh-Curtis syndrome. *N Engl J Med* 1982;306:113.
- V. Wølner-Hanssen P, Mårdh P-A, Svensson L, Weström L. Laparoscopy in women with chlamydial infection and pelvic pain: A comparison of patients with and without salpingitis. *Obstet Gynecol* 1983;61:299–303.
- VI. Wølner-Hanssen P, Weström L. Second-look laparoscopy after acute salpingitis. *Obstet Gynecol* 1983;61:702–4.
- VII. Wølner-Hanssen P, Svensson L, Mårdh P-A, Weström L. Laparoscopic findings and contraceptive usage in women with signs and symptoms suggestive of acute salpingitis. *Obstet Gynecol* 1985; 66:In Print.
- VIII. Wølner-Hanssen P, Mårdh P-A. *In vitro* tests of the adherence of *Chlamydia trachomatis* to human spermatozoa. *Fertil Steril* 1984;42:102–7.
- IX. Wølner-Hanssen P, Weström L, Mårdh P-A. Influence of amniotic fluid on the formation of chlamydial inclusions in McCoy cell cultures. In: Mårdh P-A, Holmes KK, Oriel JD, Piot P, and Schachter J (eds), *Chlamydial Infections. Proceedings of the 5th International Symposium on Human Chlamydial Infections*. Elsevier Biomedical Press, Amsterdam, New York, Oxford. 1982, pp 283–6.

ABBREVIATIONS

ESR:	Erythrocyte Sedimentation Rate
FHCS:	Fitz-Hugh-Curtis Syndrome
FITC:	Fluoroisothiocyanid
HCl:	Hydrochloric Acid
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
IUD:	Intrauterine Device
M:	Mol
NaOH:	Sodium Hydroxide
OC:	Oral Contraceptive
PBS:	Phosphate-Buffered Saline
PID:	Pelvic Inflammatory Disease
RR:	Relative Risk
2-SP:	Sucrose Phosphate Buffer
STD:	Sexually Transmitted Disease
WBC:	White Blood Cell Count

INTRODUCTION

CHLAMYDIA

Chlamydia trachomatis and *Chlamydia psittaci* are two species of the genus *Chlamydia*. All Chlamydia species are adapted to an obligate intracellular life in non-phagocytic cells (Moulder, 1974). The infectious elementary body enters the cell by phagocytosis. The ingested elementary body enlarges within a cytoplasmic vacuole to form the reticulate body. New elementary bodies differentiate from the latter by binary fission. The growth cycle of the chlamydia is completed 48–72 hours after infection by the release of elementary bodies from the host cell (Stirling and Richmond, 1977; Schachter and Caldwell, 1980).

Chlamydia trachomatis is mainly a human pathogen. As yet, 15 serotypes of *C. trachomatis* have been described (Wang & Grayston, 1971). The serotypes A, B, Ba and C cause trachoma (Grayston & Wang, 1975). This chronic infection of the conjunctivae affects an estimated 500 million people in tropical countries and is the leading cause of blindness in the world (Dawson et al., 1981). The serotypes L₁, L₂ and L₃ cause *Lymphogranuloma venereum* (LGV), a genital infection that is sexually transmitted (Grayston & Wang, 1975). LGV is most frequent in tropical countries. The serotypes D to K (Grayston and Wang, 1975) have been associated with infections in the urogenital tract such as: urethritis (Holmes et al., 1975; Bowie et al., 1977, Johannisson et al., 1977, Terho 1978) and epididymitis (Berger et al., 1978) in the male, and the infections in the female genital tract described below, but they may also cause conjunctivitis and pneumonitis in infants in nonendemic countries (Beem et al., 1977, Harrison et al., 1978, Hallberg et al., 1979, Grossman et al., 1982).

GENITAL CHLAMYDIAL INFECTIONS IN THE FEMALE

The association of *C. trachomatis* with genital infections in the female first was described by Dunlop et al. (1964). Later, several investigators have demon-

strated a relationship between *C. trachomatis* and cervicitis (Kuo et al., 1972, Hilton et al., 1974, Rees et al., 1977). Dunlop et al. (1966), Hare et al. (1981), and Paavonen et al. (1982) found that colposcopically identified follicular or hypertrophic cervicitis was associated with chlamydial infections.

Pelvic inflammatory disease (PID) is defined as an acute clinical syndrome attributed to ascending infection from the cervix to the upper genital tract (Gregg, 1982). The term PID does not specify which pelvic structures are involved in the inflammation; the fallopian tubes may or may not be infected. In contrast, acute salpingitis is a more specific definition of inflammation of the fallopian tubes after the inflammation has visually been confirmed (Jacobsson and Weström, 1969, Chaparro et al., 1979, Allen and Schoon, 1983). The annual number of new cases in Sweden has been estimated to be between 11 000 and 17 000 (Svensson, 1983). In the United States, more than 1 million cases are annually diagnosed (Blount et al., 1983). Typical sequelae after acute salpingitis are infertility (Falk, 1965; Weström, 1975; Svensson et al., 1983), tubal pregnancy, and chronic abdominal pain (Weström, 1975). This makes salpingitis the most important sexually related disease in the female.

Dunlop and co-workers (1966) suggested that there is a relationship between *C. trachomatis* infection in the cervix and acute salpingitis. This later has been verified in numerous studies, some of which are referred to below. For instance, Hamark et al. (1976) reported on 4 women with salpingitis, who harbored *C. trachomatis* in the cervix, and who developed a four-fold rise of complement fixing antibodies to the microorganism. One of the women also had *C. trachomatis* recovered from the fallopian tubes. Mårdh et al. (1977) recovered *C. trachomatis* from the fallopian tubes during laparoscopy in 6 out of 20 women with acute salpingitis. In Scandinavia, *C. trachomatis* has been isolated from the lower genital tract in 26% of 106 women (Paavonen et al. 1979), 22.3% of 166 women (Møller et al. 1981), 46.4% of 56 women

(Gjønnaess et al. 1982) and 65% of 20 women with PID (Bollerup et al. 1982). Bowie et al. (1981) in Vancouver, recovered *C. trachomatis* from the cervix in 50% of women with PID diagnosed in a STD clinic population. Sweet (1983) isolated *C. trachomatis* from 11 of 46 hospitalized women with salpingitis in San Francisco.

ROUTE OF SPREAD OF GENITAL PATHOGENS IN THE FEMALE

Falk (1946) reported on more than 1 000 women who had bilateral cornual tubal resection performed after an episode of salpingitis. None of the patients reportedly experienced recurrent PID. These findings indicate that pathogens frequently spread along the mucosal surfaces from the lower genital tract to the fallopian tubes via the uterus. Møller and Mårdh (1980) suggested in experiments with a grivet monkey model, that *C. trachomatis* can spread along the mucosa from the endometrium to the tubes causing endosalpingitis, whereas *Mycoplasma hominis* may travel to the tubal serosal layer via the parametral lymphatics or vessels, causing parametritis and ectosalpingitis (Møller et al., 1980).

If *C. trachomatis* also spreads from the cervix to the tubes via the uterine cavity in women who develop salpingitis, then isolation of *C. trachomatis* from uterine contents and histopathological evidence of endometritis would be expected in these patients. The isolation of microorganisms from the endometrium of menstruating women is possible only after transcervical collection of culture specimens. This culture method has a certain rate of contamination with cervical microorganisms even when specially designed, double lumen sampling tubes are used. Thus, false positive organism isolation may be common with this technique. The histologic definition of endometritis is unclear. This is related to the unique properties of the endometrium, i.e., infiltration with polymorphonuclear leukocytes, lymphocytes, and macrophages is physiologic during certain phases of the menstrual cycle, whereas tissue proliferation is lacking even in chronic endometritis (Brudenell, 1955). The presence of plasma cells in the endometrium has been the criterion *sine qua non* for the diagnosis of endometritis since the classical paper of Hitschmann and Adler (1907). However, there is no agreement to how many plasma cells are necessary for this diagnosis. Some pathologists tend to define endometritis when any

plasma cells are seen in the endometrial stroma (Couch, 1978), while Brudenell (1955) concludes that a slight or moderate degree of plasma cell infiltration is a common occurrence which is without significance, whereas heavy plasma cell infiltration is associated with salpingitis. Cadena et al. (1973) found no association between the number of plasma cells, and the severity of certain symptoms (irregular bleeding, pelvic pain, pelvic tenderness, fever, discharge) that might be expected in women with salpingitis. On the other hand, plasma cells may be difficult to locate in the endometrium because of small numbers, stromal distortion, the presence of a large number of other leukocytes, and the plasmacytoid appearance of stromal cells (Greenwood & Moran, 1981).

The aim of the study reported in Paper I was to investigate whether or not *C. trachomatis* is associated with endometritis among patients with salpingitis.

Periappendicitis is defined as appendiceal serositis that does not involve the intestinal mucosa. Periappendicitis was found in 12 of 189 (6.4%) (O'Neill & Moore, 1977) and in 7 of 167 (4.2%) (Butler, 1980) patients with a histologically confirmed diagnosis of appendicitis. It is of interest that 11 of the 12 patients seen by O'Neill and Moore were female. Periappendicitis was found in 12 of 27 patients with gonococcal salpingitis studied by Moritz (1912). Chlamydial salpingitis also may cause periappendicitis. Lannigan et al. (1980) and Poynard et al. (1982) each reported a case of periappendicitis associated with high titers of antibodies to *C. trachomatis*, but in both cases chlamydial cultures were negative. These data suggest that periappendicitis may result from the spread of organisms from the genital tract. Further evidence for an association between *C. trachomatis* and periappendicitis is discussed in Paper II.

Perihepatitis is an inflammatory reaction of the liver capsule and the peritoneum of the adjacent abdominal wall. The condition usually is associated with acute salpingitis. Perihepatitis first was described by Stajano in 1920. Curtis (1930) described the typical "violin-string" adhesions between the liver and the abdominal wall in patients with previous salpingitis. The condition still was widely unknown when Fitz-Hugh (1934) reported that gonococci could be isolated from the cervix of patients with perihepatitis. The syndrome later was called the Fitz-Hugh-Curtis syndrome. The syndrome typically is diagnosed by the findings of a severe, acute, right-upper abdominal pain. This pain is accentuated by deep inspiration, coughing, laughing, and twisting of

Table I. *Prevalence of perihepatitis in patients with acute salpingitis.*

Author	Year of publication	Salpingitis No.	Perihepatitis	
			No.	%
Litt and Cohen	1978	137	37	27.0
Onsrud	1980	274	38	13.9
Wang et al.	1980	523	23	4.4
Paavonen et al.	1981	341	15	4.4
Gjønnaess et al.	1982	65	18	27.7

the upper part of the body (Stanley 1946). The patients usually also have a pelvic inflammatory disease, which is often asymptomatic. The physician may be unaware of any genital tract pathology until a pelvic examination is performed. The prevalence of symptomatic perihepatitis among patients with salpingitis considerably differs in the literature. As shown in Table I, perihepatitis was reported in 4.4% of the salpingitis patients studied by Paavonen et al. (1981) and by Wang et al. (1980), whereas higher rates of perihepatitis were reported in other studies of acute salpingitis (Litt & Cohen, 1978, Gjønnaess et al., 1982, Onsrud, 1980).

Since Fitz-Hugh's publications in the 1930s, perihepatitis was thought to be a specific reaction to gonococcal infections (Amman et al., 1971, Semchishyn, 1979, von Knorring et al., 1979, Sundal & Stalder, 1980). Litt and Cohen (1978) were the first to consider other etiologic agents in this condition. They reported on 37 cases with signs and symptoms suggestive of perihepatitis, but two-thirds of the women did not have *N. gonorrhoeae* isolated from the lower genital tract. The same year, Müller-Schoop and coworkers reported on 7 women with visually confirmed perihepatitis. Three of the 7 women had significant antibody responses to *C. trachomatis* suggesting a role of this organism in perihepatitis.

CLINICAL AND LAPAROSCOPIC FINDINGS IN PATIENTS WITH PELVIC INFLAMMATORY DISEASE

Several laparoscopic studies have shown that the clinical diagnosis of salpingitis is unreliable (Jacobson and Weström, 1969, Chaparro et al., 1978, Kolmorgen et al., 1978, Allen and Schoon, 1983, Moldin and Jacobson, 1984). Laparoscopic evidence of salp-

ingitis was confirmed in only about two-thirds of women who had a clinical diagnosis of PID. These studies did not include chlamydial cultures. Svensson and coworkers (1980) demonstrated a relationship between certain clinical signs and symptoms and etiologic agents in patients with acute salpingitis. It was found that acute salpingitis caused by *C. trachomatis* is often associated with mild clinical findings, i.e., with milder pain and with less fever, than infection caused by *N. gonorrhoeae*. Thus, it is possible that a clinical diagnosis will be erroneous among women with abdominal pain and chlamydial infection, even more often than among women previously reported. It was our aim in the study reported in Paper V to compare the relationship between clinical and laparoscopic findings in patients with chlamydial infections.

EVALUATION OF ADNEXA AFTER AN EPISODE OF PELVIC INFLAMMATORY DISEASE

Laparoscopy in the acute stage of the inflammation gives but a brief glimpse of a dynamic process. The continued evolution of the inflammatory process probably is dependent on the etiologic agents involved, host defense, and the treatment regimen instituted. The efficacy of a treatment regimen usually is defined by the resolution of the acute clinical symptoms and signs or by the eradication of pathogens. The effect of various antibiotics on the post-PID infertility rate has been infrequently studied. The studies by Weström (1980) and Svensson et al. (1983) on post-PID infertility required several years of follow-up. One study (Weström et al., 1979) compared the infertility rate of women treated with four different antibiotic regimens. After a mean period of follow-up of 8 years, no difference was found, whether the patients had been treated with penicillin and streptomycin or chloramphenicol, ampicillin, or doxycycline. Falk (1965) added corticosteroids to the antibiotic regimen, but women receiving corticosteroids had no improvement of their post-PID infertility rate, after 2½ to 5 years of observation, compared to women receiving antibiotics alone. In order to find a method that would allow a more rapid evaluation of the tubes after an episode of PID, we started offering patients with visually proven salpingitis an opportunity for re-laparoscopy approximately six months after therapy. This study is reported in Paper VI.

FACTORS WITH POSSIBLE IMPACT ON THE SPREAD OF MICROORGANISMS FROM THE LOWER GENITAL TRACT

It has been estimated that approximately 8% of women infected with *C. trachomatis* in the cervix will develop salpingitis if left untreated (Bowie et al., 1981, Weström et al., 1982). This probably is an underestimation. Perhaps because chlamydial infection often causes mild symptoms, a proportion of women with salpingitis may not present for therapy. Moore et al. (1982) noted that 72% of 33 women with distally occluded fallopian tubes had antibodies to *C. trachomatis* but that only 45% of the 33 women had a history of PID. Paavonen et al. (in print) found histological evidence of endometritis among 47% of women with mucopurulent cervicitis. Little is known of the mechanism by which the microorganisms spread to the fallopian tubes. Several studies have concluded that the risk of salpingitis is reduced among oral contraceptive users, and increased among IUD-using women. Eschenbach and coworkers (1977) analyzed 173 matched pairs of patients with PID and controls. By use of the ratio of discordant pairs, they estimated the relative risk of nongonococcal PID for IUD users vs women using no contraceptives at 5.5. The relative risk for nongonococcal PID in oral contraceptive users vs nonusers of contraceptives was 0.5. Weström and colleagues (1976) compared 515 consecutive cases of laparoscopically verified salpingitis with 741 matched, healthy women who had been selected with the help of a regional population register. They concluded that the relative risk of acute salpingitis for any woman using an IUD was threefold compared with nonusers, and the risk for an oral contraceptive user was 0.2 compared with users of other contraceptives. Svensson et al. (1984) demonstrated that PID was associated with a milder tubal inflammation in oral contraceptive users compared with users of other

contraceptive methods. In Paper VII the correlation between laparoscopic findings and contraceptive use in patients with presumed PID is further discussed.

It has been postulated that spermatozoa may be vectors for bacterial transportation in the genital tract. Howard (1971) suggested that microorganisms might "hitch-hike" onto spermatozoa. There is evidence that gonococci (James et al., 1976) and *Escherichia coli* (Keith et al., 1984) attach to spermatozoa *in vitro*. Gnärpe and Friberg (1973) presented electron micrographs showing *Ureaplasma urealyticum* attached to sperm *in vivo*. Toth et al. (1982) demonstrated that various bacteria can be transported through capillary tubes in association with sperm. Whether bacteria are transported with spermatozoa *in vivo* is not known. It was the aim of one of the present studies to evaluate possible chlamydial attachment to sperm (Paper VIII).

C. trachomatis is also a common lower genital tract pathogen in pregnant women (Mårdh et al., 1980, Møller et al., 1982, Quigstad et al., 1983, Osser & Persson, 1984). Therapeutic abortion, rupture of the membranes, and labor may allow the microorganism to enter the uterine cavity. Post-abortion salpingitis (Møller et al., 1982, Quigstad et al., 1983, Osser & Persson, 1984), postpartum endometritis (Wager et al., 1980), and postpartum salpingitis (Rees et al., 1977) have been associated with *C. trachomatis* infection. Amniotic fluid can inhibit the growth of certain bacteria (Thadepalli et al., 1977) in the third trimester of pregnancy (Galask & Snyder, 1968, Schlievert et al., 1975). Individual (Schlievert et al., 1975), as well as populational (Appelbaum et al., 1977) variations in the antimicrobial properties of amniotic fluid have been demonstrated. Whether amniotic fluid has any modifying effect on pregnancy-related genital infections is unknown. In Paper IX we have reported on an *in vitro* study that focused on the influence of amniotic fluid on chlamydial growth.

AIMS OF THE INVESTIGATION

To define the route of spread of chlamydial infections in the female genital tract

- The role of *Chlamydia trachomatis* in endometritis (I)
- The role of *Chlamydia trachomatis* in periappendicitis (II)
- The role of *Chlamydia trachomatis* in perihepatitis (III, IV)

To compare clinical signs and symptoms with laboratory and laparoscopic findings in patients with presumed PID associated with chlamydial infection (V)

To describe findings at second-look laparoscopy after acute salpingitis (VI)

To evaluate factors of possible importance for the spread of *Chlamydia trachomatis* in the female genital tract

- The correlation between contraceptive usage and laparoscopic findings in women with signs and symptoms suggestive of acute PID (VII)
- *In vitro* attachment and transport of *Chlamydia trachomatis* on spermatozoa (VIII)
- The impact of amniotic fluid on chlamydial growth (IX)



MATERIALS AND METHODS

PATIENTS

The patients were derived from 1031 consecutive women who presented with a provisional diagnosis of acute pelvic inflammatory disease (PID) at the Department of Obstetrics and Gynecology, University Hospital, Lund, Sweden, from January 1975 through March 1983. Laparoscopy was performed in all patients studied. The minimum criteria for performing laparoscopy were: acute lower abdominal pain, abnormal tenderness over the adnexa at pelvic examination, abnormal vaginal discharge (i.e., polymorphonuclear leukocytes outnumbering vaginal epithelial cells in vaginal wet mounts) and/or irregular uterine bleeding. Pregnant women and women who had diseases that contraindicated laparoscopy were excluded from the studies of PID.

SPERM DONORS

The samples of human semen were delivered by healthy males who routinely were screened prior to sterilization in the Department of Forensic Medicine, University of Lund, Sweden.

AMNIOTIC FLUID DONORS

Amniotic fluid was aseptically collected by abdominal amniocentesis from healthy women in the 16–17th gestational week. The procedure was performed to screen for fetal neural tube defects or chromosomal abnormalities. The indications for amniocentesis were one or more of the following: ≥ 35 years of maternal age, a previous child with neural tube or chromosomal defects, or an anxiety for such defects to be present. For our purpose, an additional 10 milliliter of amniotic fluid was aspirated. Amniotic fluid also was collected from women at term during elective caesarian section. Oral consent to collect the fluid had been obtained.

LAPAROSCOPY

Laparoscopy was performed within 24 hours of admission. General anesthesia was induced with a barbiturate and maintained with neuroleptanalgesics. Ventilation was controlled via an endotracheal tube to compensate for muscle relaxation. A 1.5 centimeter transverse incision was placed at the lower margin of the umbilicus. An insufflation cannula was inserted through the incision. Free intraabdominal position of the tip of the cannula was determined by a "hanging drop" and aspiration technique. After the pneumoperitoneum was established with 2–3 liters of CO₂, a laparoscopic trocar was placed and the laparoscope inserted. Two manipulation cannulas were introduced through 2-mm incisions placed in each hypogastric fossa (Nordwall, 1970).

The minimum criteria for the visual diagnosis of acute salpingitis were: hyperemia of the tubal surface, edema of the tubal wall, purulent discharge from the abdominal orifice, and/or recent peritubal adhesions (i.e., easily breakable, not fibrotic adhesions). Generally, antibiotic treatment immediately was instituted after the surgery among women found to have salpingitis. Women who had no laparoscopic evidence of salpingitis nor other intraabdominal disease were given doxycycline per os from the first post-operative day.

During 1982, consecutive patients with acute salpingitis were offered a repeat laparoscopy to be performed approximately six months after therapy.

SAMPLING OF CULTURE SPECIMENS

Cervix

After insertion of a speculum into the vagina to expose the cervical os, specimens were collected for *N. gonorrhoeae* and *C. trachomatis* isolation by rotation of sampling swabs in the cervical canal.

Urethra/Rectum

Urethral and rectal specimens also were collected for *N. gonorrhoeae* culture.

Endometrium

Specimens for culture were transcervically collected by a protected aspiration method. The method was designed to avoid contamination of the endometrial specimens by the cervical flora. The vagina and cervix were washed with 0.1% chlorhexidine solution. Next, a plastic tube, fitted with a mandrin, was introduced through the cervical canal. The mandrin was replaced with a baby-feeding tube that was adapted to a syringe, and contents of the uterine cavity were aspirated. Cotton-tipped sampling sticks were soaked with the aspirated specimens were cultured for *C. trachomatis*, *N. gonorrhoeae*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*. Following the cultures, endometrial biopsies were obtained with a sharp curette and fixed in 20% formaldehyde. Endometritis was defined by the occurrence of plasma cells in the biopsy specimens.

Fallopian tubes

The abdominal orifice of the fallopian tubes was swabbed with cotton-tipped aluminum sticks that were introduced through the second puncture manipulation cannulas. In some patients, minute fimbrial biopsy specimens were collected with a punch biopsy forceps (1970). In some cases with clubbed tubes, one of the tubes percutaneously was punctured with a long needle cannula and the contents aspirated. This procedure was performed under direct laparoscopic visual control. Tubal specimens were cultured for *C. trachomatis*, *N. gonorrhoeae*, *U. urealyticum*, and *M. hominis*.

Liver surface

In patients with perihepatitis, the liver surface was swabbed with long cotton-tipped aluminum sticks. To prevent contamination of the manipulation cannula with microorganisms from the genital tract, the liver specimens always were collected before the uterus or adnexa had been touched with the instrument. The liver specimens were cultured for *C. trachomatis*, *N. gonorrhoeae*, *M. hominis*, and *U. urealyticum*.

SAMPLING STICKS AND TRANSPORT MEDIA

Specimens to be cultured for *C. trachomatis* were collected with calcium-alginate tipped aluminum swabs. After 1980, we used cotton-tipped aluminum swabs. The specimens were transported in a sucrose-phosphate buffer (2-SP) containing gentamicin (50

µg/ml), amphotericin B (2.5 µg/ml), and vancomycin (100 µg/ml).

Specimens for the isolation of *N. gonorrhoeae* and *M. hominis* were collected with charcoal-treated cotton swabs with wooden shafts, and transported in modified Stuart's medium. The specimens for the culture of *N. gonorrhoeae* alternatively were directly streaked onto Thayer-Martin blood agar plates.

CHLAMYDIAL CULTURE

C. trachomatis was cultured in cycloheximide-treated McCoy cell cultures as described by Ripa and Mårdh (1979). The McCoy cells were maintained in RPMI 1640 (Flow Labs, Ltd., Irvine, Ayrshire, Scotland), supplemented with 10% fetal calf serum, 1% glutamine, 10 µg/ml gentamicin, and 2.5 µg/ml amphotericin B. Three to four day old cell cultures were transferred to flat-bottomed plastic tubes containing a coverslip. The cultures were incubated in maintenance medium for 24 hour at 37°C to establish a monolayer on the coverslip. The maintenance medium was replaced by a mixture of 0.5 ml of transport medium containing the specimen and 0.5 ml of a culture medium (maintenance medium supplemented with 0.5% (wt/vol) of glucose and 100 µg/ml of vancomycin). The tubes were centrifuged at 4000 × g for 1 hour at 35°C. Thereafter the medium was replaced by fresh culture medium supplemented with 1–2 µg/ml cycloheximide. The cultures were incubated for 72 hours at 35–37°C. Finally, they were fixed in methanol and stained with iodine. For microscopy, the coverslips were mounted cell-side down on a microscope slide and examined at 125x magnification for the presence of chlamydial inclusions.

CHLAMYDIAL GROWTH IN PRESENCE OF AMNIOTIC FLUID

A purified strain of *C. trachomatis*, immunotype E, that had been passed in the yolk sac of embryonated hen's eggs, was tested. The chlamydial suspension was inoculated onto McCoy cell cultures and treated as mentioned above. However, for these tests Complete Medium Glucose Antibiotics (CMGA) was used as culture medium. To test possible inhibitory activity of amniotic fluid on chlamydial growth, CMGA was mixed with pooled amniotic fluid from women in the 16–17th and in the 39–41st gestational week. The concentration of amniotic fluid in the culture

medium was varied between 10 and 80 percent. Total replacement of the culture medium with amniotic fluid was also tested. To evaluate whether any reduced chlamydial growth might be due to nutritional depletion, freeze-dried amniotic fluid was mixed with CMGA in various concentrations. In a third series of tests, chlamydial elementary bodies were exposed to pooled amniotic fluid for approximately 18 hours, before they were centrifuged into a pellet and inoculated onto McCoy cells in pure CMGA medium. In other tests, pooled fluids were heated to 80°C for 20 minutes, or dialyzed before adding to infected McCoy cell cultures. After 72 hours of incubation the number of chlamydial inclusions in each culture was counted, and the percentage of inclusions in the test cultures vs control cultures calculated. The Figure in Paper IX depicts the means of these percentages from repeated tests. Pooled amniotic fluid from 50 women in the 16–17th gestational week was concentrated with ultrafiltration. The concentrate was filtrated on a Sephadex G-200 column (Pharmacia, Inc., Uppsala, Sweden), and the ultrafiltrate was freeze dried, dissolved in distilled water, and filtrated on a Sephadex G-25 column. The fractions from these gel filtrations were added into 10 (G-200) and 11 (G-25) pools, respectively. All 21 pools were freeze dried and stored at –20°C until required. Before testing the chlamydiostatic properties of the pools, distilled water was added to each of them to restore their original volume. Samples of each pool was then mixed with CMGA in various proportions and added to infected McCoy cell cultures, as mentioned above.

GONOCOCCAL CULTURE

Gonococci were isolated according to the method described by Mårdh et al. (1978). The culture plates were incubated at 37°C in an atmosphere of 7% CO₂ and 93% air in an incubator with regulation of the gas atmosphere and humidity. The plates were read under a stereomicroscope after one and two days of incubation. Gonococci were identified on the basis of colony morphology, oxidase reaction, gram staining, and sugar fermentation tests.

MYCOPLASMA CULTURE

From Stuart's transport media, inocula were transferred both into liquid and onto solid agar media (Mårdh & Weström, 1970). Liquid medium specimens were subcultured onto agar plates after 3 days

of incubation at 37°C. The plates were incubated at 37°C in an atmosphere of 90% nitrogen and 10% CO₂, and examined after 4 days under a stereomicroscope for typical colonies. If no colonies were noted, the plates were incubated and reexamined twice during the following week.

SEROLOGY

To detect serum antibodies to *C. trachomatis* a microimmunofluorescence technique, as described by Wang et al. (1974) and modified by Treharne et al. (1977), was used. Four dot-pools of chlamydial antigens were spotted onto microscope slides in clusters (pool 1: serotypes A to C; pool 2: serotypes D to K; pool 3: serotypes L₁, L₂, and L₃; pool 4: *C. psittaci*). Serial dilutions of test sera were added to the clusters of dot-pools and incubated in a moist chamber for 45 minutes at 37°C. Then the slides were rinsed in 1/15 molar of phosphate-buffered saline (PBS) for 10 minutes and allowed to dry in air. A solution of fluoroisothiocyanid (FITC)-labelled anti-human rabbit IgG was spotted onto the antigen-dots and incubated in a moist chamber for 45 minutes at 37°C. The slides were rinsed in PBS for 10 minutes, kept in deionized water for a moment, and then allowed to dry in air. After mounting with glycerine, the preparations were studied under a fluorescence microscope. Serum antibodies to gonococcal pili were detected with an indirect haemagglutination test using tannin-treated sheep erythrocytes (Reimann and Lind, 1977).

PURIFICATION AND INCUBATION OF CHLAMYDIA AND SPERMATOOZOA

Purified strains of *C. trachomatis*, immunotypes D, H and I, that had been passed in the yolk sac of embryonated hen's eggs, were tested.

Fertile men delivered ejaculates by masturbation. The specimens were pre-screened under a light microscope and only apparently normal ejaculates (i.e., with a normal concentration of motile spermatozoa) were used in our studies. In order to purify spermatozoa, the ejaculates were layered over columns of sucrose-phosphate (2-SP) that contained 7% Ficoll 400^R (Pharmacia, Inc., Uppsala, Sweden) and pelleted by centrifugation at 500 × G for 5 minutes followed by 1 200 × G for 10 minutes (Harrison, 1976). The pellet was resuspended in 2-SP, and the concentration of spermatozoa adjusted to 10⁷ cells/ml. For adherence tests, chlamydial suspensions were mixed

with purified spermatozoa and incubated at 37°C on a shaker. In order to test the impact of pH shifts of the test environment upon attachment, the pH of the test solutions was varied between 4.0 and 8.0 by adding 1/15 M, HCl or NaOH. In other tests the number of chlamydia was varied between 5×10^4 IFU/ml to 2×10^7 IFU/ml, whereas the pH was kept at 7.0 and the sperm concentration was kept unchanged at 10^7 /ml. Finally, the incubation time was varied between 5 and 50 minutes, whereas the pH was kept at 7.0, the chlamydial concentration at 2×10^7 IFU/ml, and the sperm concentration at 10^7 /ml. To remove unattached chlamydia after incubation, the mixture was centrifuged through a Ficoll solution as mentioned above for spermatozoa. The pellet was resuspended in 1/15 M phosphate buffered saline and washed twice by centrifugation.

DETECTION OF CHLAMYDIAL ATTACHMENT TO SPERMATOZOA

Immunofluorescence technique

Specimens of the incubated and washed suspension of spermatozoa and *C. trachomatis* were spotted onto glass slides, allowed to dry in air, and fixed in acetone. The slides were then overlaid with fluorescein-labelled monoclonal antibodies to *C. trachomatis* (MicroTrak^R, Chlamydia trachomatis Culture Confirmation Test, Syva Co., Palo Alto, CA, USA) and incubated for 30 minutes at 37°C in a moist chamber. The specimens were rinsed in 1/15 M PBS for 10 minutes and kept in deionized water for 10 seconds. The slides were allowed to dry in air and mounted with glycerin to be studied under a Leitz fluorescence microscope equipment ($\times 1250$).

Electron microscopy

In order to remove sucrose constituents, incubated and washed suspensions of *C. trachomatis* and spermatozoa were washed in distilled water containing

0.15% formaldehyde. The samples were stained with 2% aqueous phosphotungstic acid that was added in a proportion of 1:4. One drop of the mixture was layered on copper grids coated with carbon-platinum. Excess fluid was removed with a blotting paper. The preparations were examined in a Philips 300 electron microscope.

SPERM PENETRATION TEST

Ejaculates were incubated with purified *C. trachomatis* for 45 minutes at 37°C on a shaker. Columns of albumin prepared from fresh hen's eggs were drawn into 10 cm long capillary tubes (Vitrex^R, Swedish Hospital Supply, Gothenberg, Sweden). One end of the tubes was sealed with modeling clay. The other end was immersed into a reservoir containing the incubated mixture of *C. trachomatis* and semen. This equipment was left with the capillary tubes in a horizontal position for 3 hours at 37°C in a moist chamber. Following the incubation the tubes were cut into 2.5 cm long pieces. The part of the capillary tubes that had been immersed in the reservoir was discarded. The content of the remaining tubal pieces was spotted onto glass slides and allowed to dry in air. The slides were treated as mentioned above under immunofluorescence technique.

STATISTICAL ANALYSIS

Statistical analysis was by chi-square or Fisher's exact test for frequencies. A logistic regression analysis was used to estimate relative risks for salpingitis among users of various contraceptive methods. Two-way analysis of variance was used to compare chlamydial attachment to spermatozoa under different test conditions. Adjustments were made for multiple comparisons in Table II by multiplying the original p-values by the number of comparisons made in the table, using Bonferroni's Inequality (Miller 1966).

RESULTS AND COMMENTS

ROUTE OF SPREAD OF *C. TRACHOMATIS*
FROM THE CERVIX*Endometritis*

As shown in Table II, *C. trachomatis* was isolated from the endometrial aspirates of 12 (46%) of 26 women with laparoscopic evidence of acute salpingitis (18 of the patients were reported in Paper I). In three of the 26 patients, the endometrial, but not the cervical cultures, yielded growth to the microorganism. *N. gonorrhoeae* was recovered from the lower genital tract of 5 of the 26 women, 4 of whom also had chlamydial infection. Gonococci were isolated from the endometrial aspirate of one of the 5 patients. *M. hominis* was recovered from the cervix of 8, but not from endometrial or tubal specimens in any of the 26 women.

Endometrial aspirate was also performed in 28 women who presented with clinical signs and symptoms suggestive of PID, but did not have laparoscopic evidence of salpingitis. Six of the 28 women were infected with *C. trachomatis*; 3 harbored the organism in the endometrium (11%). In one patient the endometrial, but not the cervical specimens, and in two patients both the endometrial and the cervical specimens yielded *C. trachomatis*. These results suggest a correlation between the isolation of *C. trachomatis* from uterine contents and the presence of salpingitis ($P=0.004$). *M. hominis* was isolated from the cervix of 7 of the 28 women, but not from the endometrial aspirates of anyone. *N. gonorrhoeae* was isolated from the cervix and the endometrial aspirates in 2,

and the cervix only in one of the 28 women. The latter patient also was infected with *C. trachomatis* in the cervix and endometrium.

Endometrial biopsies for histologic examination were obtained from 11 of the 26 salpingitis and 6 of the 28 nonsalpingitis patients. Plasma cell infiltration was noted in 6 (75%) of the 8 biopsies collected from patients who harbored *C. trachomatis* in endometrial or tubal specimens, and in 2 (22%) of 9 biopsies from patients who had negative aspirate cultures for *C. trachomatis* ($P=0.04$). Thus, plasma cell endometritis was significantly correlated with growth of *C. trachomatis* in the upper genital tract. An association (though not significant in this small group) also was suggested between histologic evidence of endometritis and salpingitis, i.e., endometritis was present among 6 (55%) of 11 women with and 2 (33%) of 6 women without salpingitis ($P=0.28$). Six of 8 women with endometritis had salpingitis.

It is possible that a transcervical sampling method may result in false positive cultures because of contamination from the cervical flora. We attempted to reduce the risk of contamination in this study by using a double lumen sampling system. The absence of *M. hominis* in the endometrium among the 15 women with *M. hominis* in the cervix suggests that the sampling system may have limited contamination. The isolation of *C. trachomatis* from endometrial, but not cervical specimens, is indicative of its presence in the endometrium without cervical contamination. These results suggest that both the presence of *C. trachomatis* in the endometrium and endometritis are common among women with salpingitis and that the organism might canalicularly spread from the cervix via the uterine mucosa.

Our findings have been reconfirmed by others. Sweet (1982) was able to recover *C. trachomatis* from the endometrium of 25% of hospitalized PID patients. Paavonen et al. (1985) recently documented a significant correlation between plasma cell endometritis and laparoscopically confirmed salpingitis.

Table II. Microbial isolations from endometrial aspirates of 54 women with or without acute salpingitis.

Organism	Salpingitis		No salpingitis		p
	n = 26	(%)	n = 28	(%)	
<i>C. trachomatis</i>	12	(46)	3	(11)	0.004
<i>N. gonorrhoeae</i>	1	(4)	2	(7)	NS
<i>M. hominis</i>	0		0		—

Wasserheit et al. (submitted) demonstrated histologic evidence of endometritis in 14 of 20 women with acute salpingitis, and in one of 13 women who did not fulfill laparoscopic criteria for acute infection. A significant correlation between plasma cell infiltration of the endometrium and growth of *C. trachomatis* in endometrial aspirates from patients with PID who had been subjected to laparoscopy was also demonstrated by Paavonen et al. (submitted) and Kiviat et al. (submitted).

Periappendicitis

Seven consecutive women with acute salpingitis and histologically confirmed periappendicitis (i.e., the inflammation included only the muscularis and/or the serosa, whereas the mucosa appeared normal) are described (II). An etiological association between *C. trachomatis* and the acute salpingitis was shown by the isolation of *C. trachomatis* from all women, the presence of ≥ 4 -fold change of serum IgG antibody titer to *C. trachomatis* in 3 women, and the presence of IgM antibody to the organism in a fourth woman. None of the 7 women had *N. gonorrhoeae* recovered.

The appendix was adherent to the right oviduct in six women, and the inflammatory changes were more severe in the right tube than the left tube in five of the seven patients. These findings indicate that chlamydial infection of the fallopian tubes may directly have spread further up to the abdominal cavity, causing periappendicitis. Using an immunofluorescence technique with monoclonal antibodies to *C. trachomatis*, we attempted, without success, to detect the microorganism in sections of the appendix from each of the 7 patients.

Periappendicitis is a well known entity, but an association with *C. trachomatis* has not been demonstrated until recently (Lannigan et al., 1980; Poynard et al., 1982). Thornell and Malmgren (1985) described a patient with acute salpingitis and periappendicitis, who harbored *C. trachomatis* in the cervix. In this case, the left, but not the right, fallopian tube seemed to be involved in the inflammation. The practical implication of the association between genital infections and periappendicitis is that surgeons should be aware of salpingitis when removing an infected appendix. Antibiotics are not included in the postoperative routine treatment of patients with appendicitis. Thus, unrecognized primary salpingitis might continue untreated after surgery of a secondarily infected appendix.

Perihepatitis

We reported three cases of the so-called Fitz-Hugh-Curtis (FHC) syndrome that were associated with *C. trachomatis*, but not with *N. gonorrhoeae* (III).

Altogether, 17 (5%) of 339 women with laparoscopically confirmed salpingitis seen in the period 1978 to March 1983 also had perihepatitis. Another woman presented with right-upper abdominal pain, but perihepatitis was not confirmed when she underwent laparoscopy. The mean age of the 17 patients was 22.6 (± 5.3); 8 women were nulliparous. One of the patients used oral contraceptives, and 8 used an IUD as the methods of birth control. All but two of the patients presented with right-upper-quadrant abdominal pain. Ten of the 17 women first presented to the surgical emergency rooms, probably because they were unaware of the genital origin of the symptoms. On laparoscopy, the tubal inflammation was classified as severe in 4 (24%), moderately severe in 8 (47%), and mild in 5 (29%) women. Liver enzymes, bilirubin and pancreatic amylase serum levels were within the reference limits in 8 of 9 women studied this way. One patient had an elevated pancreatic amylase level (7.9 $\mu\text{kat/liter}$, ref. 1.2–5.0).

The 17 patients were compared with the patients from Paper V who had salpingitis without perihepatitis. This was justified because the 2 patient groups belonged to the same population and the patients had been subjected to the same procedures. Six of the patients with perihepatitis already were included in the 76 salpingitis patients presented in Paper V. Thus, 70 patients had salpingitis without perihepatitis. Comparison of the two groups revealed that patients with perihepatitis, more often than those without, had an erythrocyte sedimentation rate of ≥ 30 mm/hr on admission (Table III).

Table III. Symptoms and signs in women with salpingitis alone or with salpingitis plus perihepatitis (FHC).

Characteristics	Salpingitis (n = 70) (%)	FHC-syndrome (n = 17) (%)	p*
Irregular bleeding	53	24	NS
Increased discharge	69	59	NS
Urethritis symptoms	23	12	NS
Nausea, vomiting	30	18	NS
Diarrhea	5	20	NS
Temperature $\geq 38^\circ\text{C}$	26	12	NS
ESR > 30 mm/hr	40	82	0.0016
WBC $> 10^{10}$ /liter	45	27	NS

*The original p values have been multiplied by eight to correct for multiple comparisons

C. trachomatis was isolated from the cervix in 11, and from the endometrium only or the tubes only in one patient each. The remaining 4 patients had serologic evidence of chlamydial infection; three women had ≥ 4 -fold change of antibody titers to *C. trachomatis*, and the fourth woman had unchanged, but high IgG antibody titers to the organism (1:1024). Gonococci were not isolated from any patient. None of 3 women had gonococcal pili antibodies. The remaining 14 women were not studied this way. *M. hominis* was recovered from the cervix of only one of the 17 women. Specimens from the liver surface yielded growth of *C. trachomatis* in two patients; one of whom has been reported (IV).

Microimmunofluorescence tests for serum antibodies to *C. trachomatis* revealed high titers of IgG antibodies in most of the 17 FHC patients, one of whom presented with a titer of 1:16000, and had a titer of 1:32000 two weeks later. The geometric mean titer of serum antibodies to *C. trachomatis* was 1:1217.7 among women with and 1:66.7 among those without perihepatitis ($P < 0.0001$).

A liver biopsy taken from one patient (Wølner-Hanssen et al., 1981) showed normal liver parenchyma. The liver capsule was thicker than normal with capillary proliferation and infiltrates of polymorphonuclear leukocytes and plasma cells. This finding is similar to the histology reported by Fitz-Hugh (1934) and Husebø et al. (1978), and the liver capsule involvement without parenchymal inflammation explains the absence of liver enzyme elevation in blood specimens from most patients with perihepatitis (Amman et al., 1971, Litt & Cohen 1978, Darougar et al., 1981).

In Table IV, the correlation between contraceptive usage and the laparoscopic diagnosis is provided. There was a 0.06 risk of perihepatitis among oral con-

traceptive users compared to users of barrier methods or no method ($P < 0.005$). The risk for perihepatitis did not differ between IUD users and users of barrier methods or no contraceptives. A negative correlation between oral contraceptive usage and complication of salpingitis with perihepatitis has not been previously reported. Husebø et al. (1978) found that 8 of 14 women with acute perihepatitis used an IUD, suggesting an association between IUD usage and perihepatitis, but there was no control group in their study. Onsrud (1980), compared women who had salpingitis with and without perihepatitis and also found an association between recent IUD insertion and perihepatitis. However, oral contraceptive use was not considered in that study. The present data indicated no increased risk for perihepatitis among IUD users compared to users of barrier methods or no contraceptives.

Classically, the laparoscopic findings of perihepatitis have been described as violin-string adhesions between the liver surface and the adjacent abdominal wall. Those adhesions may represent a late stage in the disease process. Many of the patients we have examined have part of the liver surface closely adherent to the abdominal wall (Figure 1). Usually, the adhesions easily were separated. Sometimes we observed the liver adhesion slowly, but "spontaneously" being broken through the elevation of the abdominal wall during insufflation of carbon-dioxide and through the cranial dislocation of the liver on maintaining the patient in Trendelenburg's position. Discrete capillary bleeding from the abdominal wall, and a fine, fibrinous, circular ridge on the liver surface were the only remnants of the adhesions in these cases. One patient with perihepatitis and salpingitis also had filmy adhesions between the caecum and the abdominal wall and a normal appearing appendix.

Table IV. Sensitivity, specificity, and predictive values for various signs and symptoms in the diagnosis of salpingitis in women with presumed PID associated with *C. trachomatis* infection.

Characteristics	Sensitivity %	Specificity %	Predictive value when present %	Predictive value when absent %
Irregular bleeding	50	82	88	38
Nausea, vomiting	31	40	55	20
Diarrhea	5	87	50	24
Increased discharge	68	43	77	33
Rectal temp. $\geq 38^\circ\text{C}$	24	78	75	28
Palpable adnexal mass	24	79	75	28
ESR > 15 mm/hr	76	79	87	51
WBC $> 10^{10}$ /liter	44	68	78	33

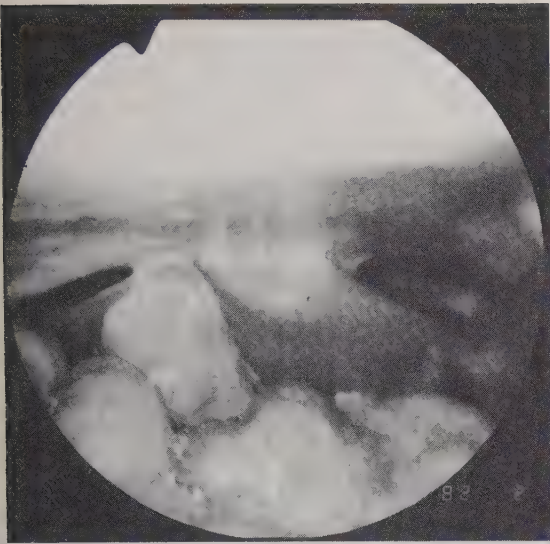


Figure 1. Anterior liver surface adhering to abdominal wall in a patient with acute perihepatitis and salpingitis

Several recent reports are in agreement with our findings; *C. trachomatis* seems to be the causative agent of perihepatitis more often than *N. gonorrhoeae* (Fransen et al., 1982, Van Beers, 1982). Of the 23 patients with perihepatitis reported by Wang et al. (1980), 16 had serologic evidence of *C. trachomatis* infection, 5 of whom also had gonococci in the cervix. Only 2 women with gonorrhea had no evidence of concurrent chlamydial infection. Paavonen et al. (1981) isolated *C. trachomatis* from 6 of 15 women with Fitz-Hugh-Curtis Syndrome. Eleven of the 15 women had serologic evidence of acute chlamydial infection. None of the 15 patients harbored *N. gonorrhoeae* in the cervix or urethra. Darougar and associates (1981) reported on 10 women with right-upper-quadrant abdominal pain, 5 of whom had perihepatitis and salpingitis proven by laparoscopy. Six of the patients had IgM antibodies to *C. trachomatis*, suggesting an acute chlamydial infection. Gjønnæss et al. (1982) found perihepatitis in 18 (28%) of 65 women with salpingitis verified by laparoscopy. *C. trachomatis* was isolated from the cervix of 7 and from the fallopian tubes of 3 of 17 women with perihepatitis. None of the 17 patients had *N. gonorrhoeae* isolated. Nine liver surface specimens were collected, but they all were sterile.

In this study, *C. trachomatis* was isolated from the liver capsule of 2 of the 17 patients with Fitz-Hugh-Curtis Syndrome. *N. gonorrhoeae* has been recovered

from the liver area previously (Fitz-Hugh, 1934, Kimball and Knee, 1970). This suggests that a spread of the microorganisms from the genital organs to the liver can take place. Whether such spread is canalicular through the abdominal cavity, or by hematogenous or lymphatic routes is unknown. The latter is suggested in a report of a man with gonococcal urethritis who apparently had perihepatitis and had *N. gonorrhoeae* recovered by liver biopsy (Kimball & Knee, 1970). Møller & Mårdh (1980) introduced *C. trachomatis* into the uterine cavity of grivet monkeys following ligature of the tubal isthmus and curettage of the endometrium. One of the monkeys died 14 days later and had peritonitis and perihepatitis at autopsy. The development of perihepatitis following tubal ligature suggests spread of the organism via lymphatic or hematogenous routes.

A canalicular spread of *C. trachomatis* from the genital tract is favored by the findings of Müller-Schoop et al. (1978) who found that peritonitis was present in all 7 women with perihepatitis. An experimental study by Schildt and Eiseman (1960) also may support a hypothesis of canalicular spread in the abdominal cavity. They demonstrated that particulate matter introduced in the cul-de-sac collects beneath the diaphragm within a few minutes. The transport of the material along the serous peritoneal surface, covered with a film of intraperitoneal fluid, probably obey the laws of capillary attraction.

Wang and co-workers (1980) suggested that chlamydial perihepatitis might be the result of an immunological reaction induced by a genital reinfection with a heterologous immunotype of *C. trachomatis*. They based their hypothesis on the fact that the immunotype specificity of IgM antibodies differed from that of IgG antibodies in 7 of 11 patients with chlamydial perihepatitis. However, only one of 15 patients reported by Paavonen et al. (1981) seemed to have been reinfected with a different immunotype of *C. trachomatis*. In experimental *C. trachomatis* infection of monkeys, conjunctival reinfection with heterologous immunotypes lead to more severe inflammatory changes compared to reinfection with homologous strains (Wang & Grayston, 1971). Patton et al., (1985), infected the tubes of a pig-tailed macaque with *C. trachomatis*. The infection first caused salpingitis, and produced perihepatitis after rechallenge of the tubes with different strains of *C. trachomatis*. In a study on cell-mediated immunity to *C. trachomatis* among women with perihepatitis and/or salpingitis, lymphocytes from both groups

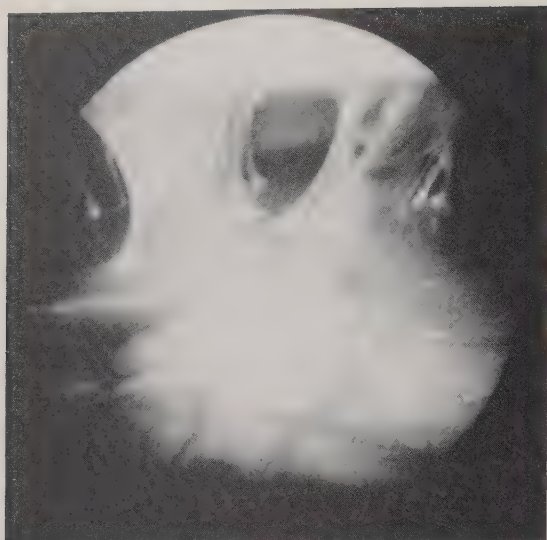


Figure 2. Filmy and violin-string adhesions between the liver and abdominal wall after perihepatitis

showed a similar response to chlamydial antigens in the acute phase, whereas lymphocytes from women with perihepatitis tended to maintain a higher level of responsiveness at follow-up visits compared to lymphocytes from patients who only had salpingitis (Hallberg et al., in print). These data also can be interpreted to suggest that perihepatitis is a result of a hyperimmune reaction to local chlamydial colonization.

The different reported rates of perihepatitis among women with salpingitis may in part be explained by differences in the diagnostic approach. Some authors only presented cases that had been verified by laparoscopy (Gjønnaess et al., 1982), whereas others mainly based their diagnosis on the findings of upper quadrant pain (Litt & Cohen, 1978, Wang et al., 1980, Paavonen et al., 1981). Laparoscopic findings of filmy or violin-string adhesions alone (Figure 2) do not prove the presence of acute perihepatitis, since such adhesions may remain as sequelae after a previous episode of perihepatitis (Grossman et al., 1981). Acute perihepatitis is characterized by easily breakable adhesions, local peritonitis and/or fibrinous plaques on the liver surface. The clinical appearance of perihepatitis easily might be confused with other upper abdominal conditions, such as gallbladder disease (Trimble, 1970, Darougar et al., 1981, Wood et al., 1982). On the other hand, the frequent referral of patients with Fitz-Hugh-Curtis Syndrome to surgical

emergency rooms would result in an underestimation of the prevalence of perihepatitis among women with salpingitis who are studied by gynecologists.

Patients with acute right-upper abdominal pain suggestive of perihepatitis should be hospitalized and treated with antibiotics active against *C. trachomatis* and *N. gonorrhoeae*. If laparoscopy is performed, the liver adhesions probably should be divided. Secondary laparoscopic division of the adhesions also might be considered in cases of chronic postinflammatory right-upper abdominal pain. This approach caused dramatic resolution of symptoms among 4 patients reported by Reichert & Valle (1976).

CLINICAL VS LAPAROSCOPIC FINDINGS IN PATIENTS WITH CHLAMYDIAL INFECTION

We reported on an analysis of 104 consecutive women who had been subjected to laparoscopy because of clinical signs and symptoms suggestive of acute salpingitis and were found to have *C. trachomatis* (V). Women who were infected with *N. gonorrhoeae* were excluded from the study. *M. hominis* was recovered from the cervix of 16 women.

Laparoscopic findings

Laparoscopy revealed acute salpingitis in 76 and visually normal tubes in 28 of the 104 women. Five of the 28 patients without salpingitis had abnormal intraabdominal conditions, such as mild endometriosis (2), endometriosis plus corpus luteum hematoma (1), corpus luteum hematoma alone (1), or mesenterial lymphadenitis (1).

Clinical findings

Women with and without salpingitis were compared for the occurrence of various clinical signs and symptoms and laboratory findings. In this comparison patients with salpingitis had a longer history of pain before seeking medical care, more often had irregular uterine bleeding, and an elevated ESR, compared with patients without salpingitis. However, when adjusting for multiple comparisons, only the presence of elevated ESR significantly differ between the 2 groups ($P < 0.006$). Table IV shows the sensitivity, specificity, and predictive values for various clinical signs and symptoms in the diagnosis of salpingitis. Elevated ESR and a history of irregular uterine bleeding had the highest predictive values. Elevated rectal temperature and palpable adnexal masses, two signs

often mentioned in textbooks as typically associated with PID, had low predictive values among these patients. The combination of elevated ESR and a history of metrorrhagia had a sensitivity of 33%, specificity of 93%, and a predictive value of 94%, whereas the combination of elevated ESR, a history of metrorrhagia, and elevated rectal temperature had a sensitivity of 11%, specificity of 100%, and predictive value of 100%.

To evaluate whether the number of signs and symptoms could be used to predict the presence of salpingitis, we compared this number in the two groups. Patients with salpingitis had a greater number of symptoms than patients without salpingitis, but there was a great deal of overlap between the groups. At least three symptoms were necessary to allow more than a 75% chance of correctly predicting tubal involvement in the disease. However, 20% of the patients with salpingitis only had one or two symptoms, and over half of the patients without salpingitis had three or more symptoms. This analysis demonstrates that, in an individual case, it is difficult to determine whether or not the tubes are involved in the infectious process by use of clinical criteria only. This is in concordance with several previous studies which demonstrated that salpingitis is difficult to diagnose by clinical criteria (Jacobson & Weström, 1969, Chaparro et al., 1978, Kolmorgen et al., 1978, Allen & Schoon, 1983, Moldin & Jacobson, 1984). None of the studies focused on chlamydial infections.

Normal laparoscopic findings in patients presenting with acute lower abdominal pain, adnexal tenderness, and sometimes even elevated WBC, ESR, and rectal temperature suggest that mild cases of PID may remain undetected after laparoscopy. Thus, it is possible that some women with visually normal tubes had endosalpingitis, and since an endometrial biopsy not routinely was performed, endometritis would remain unrecognized. One also might speculate whether the patients had parametritis. Inflammation of the parametria might explain the presence of adnexal tenderness in women with visually normal tubes and ovaries. However, no data are available that show whether or not parametritis occurs in association with chlamydial PID. Further studies are needed that include endometrial and tubal biopsy in patients without salpingitis, to define the sensitivity of laparoscopy for the diagnosis of PID. Recent preliminary results showed that 2 of 14 women with clinically presumed PID on the basis of abdominal pain and adnexal tenderness, who did not fulfill the laparo-

scopic criteria for salpingitis, had plasma cell endometritis (Kiviat et al., submitted).

For the immediate patient care, it might be unimportant whether women with *C. trachomatis* or *N. gonorrhoeae* have visually confirmed salpingitis, because they would be treated even if the tubes were normal. However, women without either organism who present with abdominal pain, are often diagnosed to have a condition other than salpingitis. Laparoscopy offers them an accurate diagnosis. In addition, the risk of infertility has been shown to be related to the degree of tubal abnormality visualized during the acute phase (Weström 1975). As yet, laparoscopy is the only diagnostic method available that can give us information on the degree of tubal involvement, and on the microorganisms present in the tubes in patients with PID.

SECOND LOOK LAPAROSCOPY

Patients with laparoscopically verified acute salpingitis were offered a repeat laparoscopy four to eight months after the initial examination. We have reported on 13 patients studied with second-look laparoscopy (VI). Nine of the 13 women had mild salpingitis and five women had moderately severe disease at the acute examination. Eleven of the cases were associated with chlamydial infection, four of whom had *M. hominis* in the cervix, and three also had gonorrhea. One woman had *M. hominis* only in the cervix. The patients were treated with doxycycline, tetracycline or rosaramicin immediately after the initial laparoscopy. None of the aforementioned microorganisms were recovered from the cervix at the time of relaparoscopy.

At repeat laparoscopy, 8 of 10 patients had one or both adnexa free of initially observed adhesions. In two patients adnexal adhesions had occurred after the initial examination. Obvious secondary tubal occlusion was seen in one patient. Chromopertubation was not performed in this study. Thus, the occurrence of proximal tubal occlusion would remain undetected.

In a recent study 9 of 20 patients, who underwent second-look laparoscopy and concomitant chromopertubation, had adhesions and/or tubal occlusions (Paavonen et al., unpublished data). The present study suggests that adhesions, at least when fresh, might be resorbed and completely disappear. Adequate antibiotic treatment in an early stage of the disease may be necessary for such resorption to occur. Second-look laparoscopy could be used to evaluate

the efficacy of various antibiotic treatment regimens for acute salpingitis, 6 to 12 months after infection. At present, investigations to evaluate post-PID fertility need to wait several years following therapy.

FACTORS WITH POSSIBLE IMPACT ON BACTERIAL SPREAD FROM THE LOWER GENITAL TRACT

Contraception

We reported on 738 women with acute signs and symptoms suggestive of salpingitis (VII). Laparoscopy showed evidence of acute tubal inflammation in 544 (73.3%) women, whereas 195 (26.3%) had visually normal fallopian tubes.

The laparoscopic findings were correlated with the contraceptive method used by the patient at the time the abdominal pain first occurred. Acute salpingitis was noted in 59.8% of the 286 oral contraceptive users, in 80.6% of the 227 IUD users, and in 84.4% of the 225 women using barrier methods, or no contraceptives (Reference Group). The relative risk of salpingitis for oral contraceptive users vs the Reference Group was 0.24 ($P < 0.0001$). This calculation included adjustment for possible confounding factors such as age and duration of pelvic pain. These relationships also were found within the subgroups of women who were infected with *C. trachomatis* and/or *N. gonorrhoeae*. That is, the risk of tubal involvement in the infection among oral contraceptive users as opposed to the Reference group was 0.22 for women with *C. trachomatis* ($P = 0.005$) and 0.06 for women with *N. gonorrhoeae* ($P = 0.001$). There was no significant difference in the accuracy of the clinical diagnosis of salpingitis between women in the IUD and the Reference groups. However, it must be emphasized that the patients studied were selected because they presented with presumptive PID. Thus, lack of laparoscopic difference between the IUD group and the Reference group does not imply that there is no difference between the groups with regard to the risk of acquiring PID.

The study indicates that oral contraceptive users with a genital tract infection are to some degree protected against spread of the infection to the fallopian tubes. This finding is in concordance with several previous epidemiologic studies showing that oral contraceptive users less often contract PID, whereas IUD users have an increased risk of the disease (Weström

et al., 1976, Faulkner & Ory, 1976, Eschenbach et al., 1977). In the present study, twice as many oral contraceptive users as IUD users, and almost three times as many oral contraceptive users as users of barrier methods/no contraceptives had no laparoscopic signs of salpingitis, even when the diagnosis was clinically suspected. This may mean that previous studies based on a clinical diagnosis of salpingitis without a laparoscopic confirmation, may have over-represented the proportion of women using oral contraceptives among the cases. Thus, the true risk of salpingitis among oral contraceptive users may even be lower than that already reported. The protective effect of oral contraceptives on the development of salpingitis seems even more striking, when one considers a possible increased risk of chlamydial infections in the lower genital tract among oral contraceptive users compared to women using other contraceptives (Ripa et al., 1978, Svensson et al., 1981, Paavonen & Vesterinen, 1982).

A recent paper (Svensson et al., 1984) considered the possible correlation between contraceptive use and degree of tubal inflammation in salpingitis patients. Most of the patients in this report also were presented in Paper VII. Oral contraceptive users had a milder disease than IUD users or women in the Reference group. Moreover, oral contraceptive users who had chlamydia-associated salpingitis did not develop infertility due to postinflammatory tubal damage, as opposed to patients using other birth control methods (Svensson et al., 1983).

The mechanisms responsible for the observed protective effects of oral contraceptives in both reducing the rate and severity of salpingitis are not known. Oral contraceptive use is associated with dense cervical mucus (Moghissi et al., 1973). The mucus plug is impenetrable for spermatozoa and thus constitutes one of the contraceptive effects of oral contraceptives. This plug theoretically also might provide a barrier for bacteria. However, it is unlikely that these uterine factors can cause the aforementioned negative correlation between oral contraceptive use and tubal damage or perihepatitis among patients with chlamydia-associated salpingitis (Table V).

There is some evidence that tissue damage associated with chlamydial infections may be caused by cytotoxic cells (Young & Taylor, 1984). Laemmert (1982) reported that spleen cells from infected mice are toxic for macrophages infected with *C. trachomatis*. Several studies have demonstrated a significantly depressed lymphocyte response to *Phytohae-*

Table V. Birth control methods used by patients with salpingitis alone or with salpingitis plus perihepatitis (FHC).

Disease	Contraceptive method		
	OC	IUD	Reference
Salpingitis	29	26	15
FHC	1	8	8

RROC vs reference 0.06 (95% confidence interval = 0.007–0.6, $X^2_1 = 9.13$, $p < 0.005$); RRIUD vs reference: 0.58 ($X^2 = 0.86$)

magglutinin (PHA) among women taking oral contraceptives (Hagen & Frøland, 1972, Fitzgerald et al., 1973, Barnes et al., 1974). Paaavonen et al. (1981) demonstrated that estradiol can inhibit suppressor T-cell activity *in vitro*. Moreover, the geometric mean titers of IgG antibody to *C. trachomatis* in women with chlamydial salpingitis were significantly ($P = 0.003$) lower among users of oral contraceptives (1:25) versus non-users (1:109) (Wølner-Hanssen, submitted). One might speculate that the reduced inflammatory response to genital infections in oral contraceptive users is caused by inhibition of the immune system.

Spermatozoa

We have demonstrated that *C. trachomatis* might attach to human spermatozoa *in vitro* (VIII). Attachment was enhanced when the pH in the test media was reduced from 8 to 4, when the concentration of *C. trachomatis* vs spermatozoa in the incubation mixture was increased, or when the incubation time was increased from 5 to 60 minutes. Attached chlamydia were transported by the spermatozoa through albumen. Albumen has properties similar to preovulating cervical mucus. The clinical implications of these findings are unknown. Whether elementary bodies attach to spermatozoa in the cervix in case of cervicitis, or attach to sperm in the reproductive tract of an infected male has not been studied. The relatively high pH in cervical mucus and semen may inhibit such attachment. Whether an infectious dosage large enough to produce salpingitis can be transferred up to the tubes may be a factor of pathogenic significance. This critical infectious dosage is not known.

AMNIOTIC FLUID

Amniotic fluid inhibited the formation of chlamydial inclusions in a concentration-dependent manner. The tests using freeze-dried amniotic fluid or pre-incubation of EBs with whole amniotic fluid, demonstrated that the inhibition was caused by a factor in the fluid, and not by a nutritional deficit. The inhibitory factor seemed to be directed against *C. trachomatis* and not against the McCoy cells, since no cytopathic effect was observed in the latter after exposure to amniotic fluid without adding EBs. Finally, the active factor in amniotic fluid was heat resistant and did not readily pass the 25 Å pores in the dialysis tubing. There was no difference in the chlamydiostatic activity of amniotic fluid from the 39–41st as compared with the 16–17th gestational weeks.

We did not find convincing inhibitory activity on chlamydial growth when we separately tested each fraction of gel filtrated amniotic fluid. After mixing all fractions to restore amniotic fluid, a similar inhibitory activity, as seen with unprocessed fluid was demonstrated (Wølner-Hanssen, Løgdeberg, Mårdh, previous unpublished data). These findings suggest that the chlamydiostatic property of amniotic fluid might be dependent on more than one component.

Larsen et al. (1974) tested the impact of amniotic fluid on the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus faecalis*. The inhibitory activity demonstrated was associated with zinc together with a peptide (Schlievert et al., 1976). According to Schlievert et al. (1977) this peptide was absent in half of the fluids from the 20th gestational week. The authors could not demonstrate a bacteriostatic effect in fluids lacking the peptide. In our hands, however, such second trimester fluids were equally inhibitory to *C. trachomatis* as were full-term fluids. These discordant findings might indicate that other components are responsible for the chlamydiostatic effect. Lysozyme, transferrin, 7 S immunoglobulin (Galask & Snyder, 1970), and peroxidase (Larsen et al., 1974) are other components known to have antimicrobial properties. Whether the inhibitory activity of amniotic fluid on *C. trachomatis* has any clinical implication is unknown. Further studies are necessary to define the inhibitory factor(s) in such fluid and to evaluate their role in host defence.

SUMMARY

Histopathologic evidence of endometritis and isolation of *C. trachomatis* from endometrial aspirates in patients with acute salpingitis, indicate that the microorganism can spread from the endocervix to the fallopian tubes via the endometrium (I).

Periappendicitis in patients with chlamydial salpingitis suggest further spread of the microorganism from the fallopian tubes to the appendix. Adherence of the appendix to the right fallopian tube in patients with periappendicitis suggests direct spread of *C. trachomatis* from the adjacent tube.

Laparoscopic evidence of perihepatitis in patients with chlamydia-associated salpingitis, and the lack of evidence of gonorrhoea in any of the FHC patients recently seen in our Department indicates that *C. trachomatis* may be a more important etiologic agent associated with the syndrome than *N. gonorrhoeae* (III). Consequently, the term "gonococcal perihepatitis" should no longer be used, unless a gonococcal etiology is demonstrated.

Isolation of *C. trachomatis* from the liver surface in FHC shows that the syndrome can be caused by spread of the microorganism from the fallopian tubes (IV).

The clinical diagnosis of acute salpingitis in patients with chlamydial infection is unreliable (V).

Second-look laparoscopy after treatment of acute salpingitis indicates that periadnexal adhesions may be resorbed and that progress of the inflammatory alterations in the tubes after adequate antibiotic treatment may be unusual (V).

Oral contraceptive users with clinical signs and symptoms suggestive of PID have a significantly reduced risk of tubal involvement as compared with users of other contraceptives (VII). This indicates that oral contraceptives can modify the manifestations of PID.

In vitro attachment of *C. trachomatis* to human spermatozoa supports the hypothesis that the latter may act as vectors for microorganisms in the genital tract (VIII).

Amniotic fluid contains factors that inhibit chlamydial growth (IX). The significance of this finding *in vivo* is unknown. This property of the amniotic fluid may be relevant to the spread of cervical chlamydial infections in pregnant women.

ACKNOWLEDGEMENTS

I wish to thank:

My teachers and friends, Professor Per-Anders Mårdh, M.D., and Associate Professor Lars Weström, M.D., for their invaluable scientific guidance; the greatest share of credit for the accomplishment of this work must be given to them;

Professors Birger Åstedt, M.D., and Rune Grubb, M.D. for their positive attitude to my work, and for permitting me the use of facilities at the Department of Obstetrics and Gynecology, and the Department of Medical Microbiology, University of Lund;

My wife, Camilla Wølner-Hanssen, R.N., for excellent technical assistance and for never failing loyalty and support;

Professors King K. Holmes, M.D., Ph.D., and David A. Eschenbach, M.D., University of Washington, Seattle, for invaluable support and stimulating discussions;

My co-workers, Lennart Lögdeberg, M.D., Birger R. Møller, M.D., and Lars Svensson, M.D., for stimulating collaboration;

Miss Eva Sandgren and Mrs. Christina Pehrson for invaluable technical assistance;

Mr. Jarl Ekstrand for generous collaboration and for teaching me in the field of electron microscopy;

My colleagues and the staff at the Department of Obstetrics and Gynecology, and at the Department of Medical Microbiology for their positive attitude and help;

Mrs. Cathy Critchlow, M.S., and Miss Laura Koutsky, MSPH, University of Washington, Seattle, for expert statistical advice;

Mrs. Ferne Beier, University of Washington, Seattle, for efficient secretarial assistance.

This work was supported by grants from W.H.O., the Swedish Medical Research Council (Grant No. 16 X-04509), Royal Physiographic Society in Lund, and the Faculty of Medicine, University of Lund.

REFERENCES

1. Allen LA, Schoon MG. Laparoscopic diagnosis of acute pelvic inflammatory disease. *Br J Obstet Gynecol* 1983; 90:966–8.
2. Amman R, Zehender O, Jenny S, Bass G. Die Perihepatitis acuta gonorrhoeica (Fitz-Hugh-Curtis-Syndrome). *Deutsche Med Wschr* 1971; 96:1515–19.
3. Appelbaum PC, Holloway Y, Ross SM, Dhupelia I. The effect of amniotic fluid on bacterial growth in three population groups. *Am J Obstet Gynecol* 1977; 128:868–71.
4. Barnes EW, MacCuish AC, Loudon NB, Jordan J, Irvine WJ. Phytohaemagglutinin-induced lymphocyte transformation and circulating autoantibodies in women taking oral contraceptives. *Lancet* 1974; i:898–900.
5. Beem MO, Saxon EM. Respiratory-tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N Engl J Med* 1977; 296:306–10.
6. Berger RE, Alexander ER, Monda GD, Ansell J, McCormick G, Holmes KK. *Chlamydia trachomatis* as a cause of acute "idiopathic" epididymitis. *N Engl J Med* 1978; 298:301–4.
7. Blount JH, Reynolds GH, Rice RJ. Pelvic inflammatory disease: Incidence and trends in private practice. Morbidity and Mortality Weekly Report, U. S. Department of Health and Human Services, Centers for Disease Control, Atlanta, Georgia, 1983; 32(4SS): 27SS–34SS.
8. Bollerup AC, Kristensen GB, Mårdh P-A, Lind I. Laboratory diagnosis of *Chlamydia trachomatis* infection in patients with acute salpingitis. In: Mårdh P-A, Holmes KK, Oriel JD, Piot P, and Schachter J (eds). *Chlamydial Infections*. Proceedings of the 5th International Symposium on Human Chlamydial Infections. Elsevier Biomedical Press, Amsterdam, New York, Oxford 1982, pp. 171–4.
9. Bowie WR, Wang S-P, Alexander ER, et al. Etiology of non-gonococcal urethritis. Evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *J Clin Invest* 1977; 59:735–42.
10. Bowie WR, Jones H. Acute pelvic inflammatory disease in outpatients: association with *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Ann Intern Med* 1981; 95:685–8.
11. Brudenell JM. Chronic endometritis and plasma cell infiltration of the endometrium. *J Obstet Gynecol Br Emp* 1955; 62:269–74.
12. Butler C. Surgical pathology of acute appendicitis. *Human Pathol* 1981; 12:870–8.
13. Cadena D, Cavanzo FJ, Leone CL, Taylor HB. Chronic endometritis. A comparative clinicopathologic study. *Obstet Gynecol* 1973; 41:733–8.
14. Chaparro MV, Ghosh S, Nashed A, Poliak A. Laparoscopy for the confirmation and prognostic evaluation of pelvic inflammatory disease. *Int J Gynecol Obstet* 1978; 15:307–9.
15. Couch RD. Chronic endometritis — medicine or mythology. *Am Soc Clin Pathol Summary Rep* 1978; 15:1.
16. Curtis AH. A cause of adhesions in the right upper quadrant. *JAMA* 1930; 94:1221–2.
17. Darougar S, Forsey T, Wood JJ, Bolton JP, Allan A. Chlamydia and the Curtis-Fitz-Hugh syndrome. *Br J Vener Dis* 1981; 57:391–4.
18. Dawson CR, Jones BR, Tarizzo ML. Guide to trachoma control. Geneva: World Health Organization 1981; 7.
18. Dunlop EMC, Jones BR, Al-Hussaini MK. Genital infection in association with TRIC virus infection of the eye. *Br J Vener Dis* 1964; 40:33–42.
19. Dunlop EMC, Harper JA, Al-Hussaini MK, et al. Relation of TRIC agent to "nonspecific" genital infection. *Br J Vener Dis* 1966; 42:77–87.
20. Eschenbach DA, Buchanan TM, Pollock HM, et al. Polymicrobial etiology of acute pelvic inflammatory disease. *N Engl J Med* 1975; 293:166–71.
21. Eschenbach DA, Harnisch JP, Holmes KK. Pathogenesis of acute pelvic inflammatory disease: role of contraception and other risk factors. *Am J Obstet Gynecol* 1977; 128:838–50.
22. Falk V. Treatment of acute non-tuberculous salpingitis with antibiotics alone and in combination with glucocorticoids. *Acta Obstet Gynecol Scand* 1965; 44(Suppl) 6:1–118.
23. Falk HC. Interpretation of the pathogenesis of pelvic infection as determined by corneal resection. *Am J Obstet Gynecol* 1946; 52:66–73.
24. Faulkner WL, Ory HW. Intrauterine devices and acute pelvic inflammatory disease. *JAMA* 1976; 235: 1851–3.
25. Fitz-Hugh T. Acute gonococcal peritonitis of the right upper quadrant in women. *JAMA* 1934; 102:2094–6.
26. Fitzgerald PN, Pickering AF, Ferguson DN. Depressed lymphocyte response to PHA in long-term users of oral contraceptives. *Lancet* 1973; i:615.
27. Fransen L, Avonts D, Piot P. Genital chlamydial infection associated with perihepatitis (Fitz-Hugh-Curtis syndrome). *Acta Clin B* 1982; 37:314–7.

28. Galask RP, Snyder IS. Bacterial inhibition by amniotic fluid. *Am J Obstet Gynecol* 1968; 102:949–55.
29. Galask RP, Snyder IS. Antimicrobial factors in amniotic fluid. *Am J Obstet Gynecol* 1970; 106:59–65.
30. Gjønnaess H, Dalaker K, Ånestad G, Mårdh P-A, Kvile G, Bergan T. Pelvic inflammatory disease. Etiological studies with emphasis on chlamydial infection. *Obstet Gynecol* 1982; 59:550–5.
31. Gnärpe H, Friberg J. T mycoplasmas on spermatozoa and infertility. *Nature* 1973; 245:97–8.
32. Grayston JT, Wang S-P. New knowledge of chlamydiae and the disease they cause. *J Infect Dis* 1975; 132:87–105.
33. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observations. *Obstet Gynecol* 1981; 58:176–84.
34. Gregg MB, editor. Morbidity and Mortality Weekly Report, U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, Georgia, 1982; 31:438.
35. Grossman MJ, Rosenfeld DL, Bronson RA. Perihepatic adhesions in infertility patients with prior pelvic inflammatory disease. *J Reprod Med* 1981; 26: 625–6.
36. Grossman M, Schachter J, Sweet R, Bishop E, Jordan C. Prospective studies in chlamydia in newborns. In: Mårdh P-A, Holmes KK, Oriel JD, Piot P, and Schachter J (eds). *Chlamydial Infections. Proceedings of the 5th International Symposium on Human Chlamydial Infections*. Elsevier Biomedical Press, Amsterdam, New York, Oxford 1982, pp. 213–16.
37. Hagen C, Frøland A. Depressed lymphocyte response to PHA in women taking oral contraceptives. *Lancet* 1972; *i*:1185.
38. Hallberg T, Wølner-Hanssen P, Mårdh P-A. Pelvic inflammatory disease in patients infected with *Chlamydia trachomatis*: cell-mediated immune response to chlamydial antigens *in vitro*. *Genitourinary Med* 1985, in print.
39. Hallberg A, Mårdh P-A, Persson K, Ripa KT. Pneumonia associated with *Chlamydia trachomatis* infection in an infant. *Acta Paediatr Scand* 1979; 68.
40. Hamark B, Brorsson J-E, Eilard T, Forssman L. Salpingitis and chlamydiae subgroup A. *Acta Obstet Gynecol Scand* 1976; 55:377–8.
41. Hare MJ, Toone E, Taylor-Robinson D, et al. Follicular cervicitis — colposcopic appearances and association with *Chlamydia trachomatis*. *Br J Obstet Gynecol* 1981; 88:174–80.
42. Harrison RAP. A highly efficient method for washing mammalian spermatozoa. *J Reprod Fert* 1976; 48: 347–53.
43. Harrison HR, English MG, Lee CK, Alexander ER. *Chlamydia trachomatis* infant pneumonitis: Comparison with matched controls and other infant pneumonitis. *N Engl J Med* 1978; 298:702–8.
44. Hilton AL, Richmond SJ, Milne JD, et al. Chlamydia A in the female genital tract. *Br J Vener Dis* 1974; 50:1–10.
45. Hitschmann F, Adler L. *Z Geburtsh Gynäk* 1907; 60: 63–86.
46. Holmes KK, Handsfield HH, Wang S-P, et al. Etiology of nongonococcal urethritis. *N Engl J Med* 1975; 292:1199–1205.
47. Howard TL. Bacterial hitch-hikers. *J Urol* 1971; 106:94.
48. Husebø OS, Bjerkeseth T, Kalager T. Acute perihepatitis. *Acta Clin Scand* 1979; 145:483–5.
49. Jacobson L, Weström L. Objectivized diagnosis of pelvic inflammatory disease. *Am J Obstet Gynecol* 1969; 105:1088–98.
50. James AN, Wende RD, Williams RP. Attachment of gonococci to sperm: Influence of physical and chemical factors. *Br J Vener Dis* 1976; 52:128–35.
51. Johansson G, Edmar B, Lycke E. *Chlamydia trachomatis* infection and venereal disease. *Acta Dermatovener* 1977; 57:455–8.
52. Keith LG, Berger GS, Edelman DA, et al. On the causation of pelvic inflammatory disease. *Am J Obstet Gynecol* 1984; 149:215–24.
53. Kimball MW, Knee S. Gonococcal perihepatitis in a male. *N Eng J Med* 1970; 282:1082–3.
54. Kiviat NB, Wølner-Hanssen P, Eschenbach DA, et al. Histopathology of endometritis and salpingitis caused by *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*. Submitted.
55. Kolmorgen K, Seidenschnur G, Weigen G. Diagnostik und Therapie des akuten Adnexprozesses beim Einsatz der Laparoskopie. *Zbl Gynaekol* 1978; 100: 1103–9.
56. Kuo C-C, Wang S-P, Wentworth BB, et al. Primary isolation of TRIC organisms in HeLa 229 cells treated with DEAE-Dextran. *J Infect Dis* 1972; 125:665–8.
57. Laemmert JK. Cytotoxic cells induced after *Chlamydia psittaci* infection in mice. *Inf Immun* 1984; 35: 1011–17.
58. Lannigan R, Hardy G, Tanton R, Marrie TJ. *Chlamydia trachomatis* peritonitis and ascites following appendectomy. *Can Med Assoc J* 1980; 123:295–6.
59. Larsen B, Galask RP, Snyder IS. Muramidase and peroxidase activity of human amniotic fluid. *Obstet & Gynecol* 1974; 44:219–23.
60. Litt IF, Cohen MI. Perihepatitis associated with salpingitis in adolescents. *JAMA* 1978; 240:1253–4.
61. Mårdh P-A, Weström L. Tubal and cervical cultures in acute salpingitis with special reference to *Mycoplasma hominis* and T-strain mycoplasmas. *Br J Vener Dis* 1970; 30:510–17.
62. Mårdh P-A, Ripa T, Svensson L, Weström L. *Chlamydia trachomatis* infection in patient with acute salpingitis. *N Engl J Med* 1977; 269:1377–9.
63. Mårdh P-A, Mårtensson D, Soltesz LV. An effective, simplified medium for the culture of *Neisseria gonorrhoeae*. *Sex Transm Dis* 1978; 5:10–13.
64. Mårdh P-A, Helin I, Bobeck S, Laurin J, Nilsson T. Colonization of pregnant and puerperal women and newborns with *Chlamydia trachomatis*. *Br J Vener Dis* 1980; 56:96–100.
65. Mårdh P-A, Möller BR, Ingerslev HJ, Nüssler E, Weström L, Wølner-Hanssen P. Endometritis caused by *Chlamydia trachomatis*. *Br J Vener Dis* 1981; 57:191–5.

66. Miller R, Jr. Simultaneous statistical inference. New York, McGraw Hill, 1966, p. 67.
67. Moghissi KS, Syner FN, McBride LC. Contraceptive mechanism of action of microdose norethindrone. *Obstet Gynecol* 1973; 41:585–94.
68. Moldin P, Jacobson M. Gynekologisk laparoskopi i rutinsjukvården — en prospektiv studie. *Läkartidningen* 1984; 81:4045–8.
69. Møller BR, Freundt EA, Mårdh P-A. Experimental pelvic inflammatory disease in grivet monkeys provoked by *Chlamydia trachomatis* and *Mycoplasma hominis*. *Am J Obstet Gynecol* 1980; 138:990–5.
70. Møller BR, Mårdh P-A. Experimental salpingitis in grivet monkeys. Modes of spread of infection to the fallopian tubes. *Acta Path Microbiol Scand, Sect B* 1980; 88:107–14.
71. Møller BR, Mårdh P-A, Ahrons S, Nüssler E. Infection with *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Neisseria gonorrhoeae* in patients with acute pelvic inflammatory disease. *Sex Transm Dis* 1981; 8:198–202.
72. Møller BR, Ahrons S, Laurin J, Mårdh P-A. Pelvic infection after elective abortion associated with *Chlamydia trachomatis*. *Obstet Gynecol* 1982; 59:210–11.
73. Moore DE, Spadoni LR, Foy HM, et al. Increased frequency of serum antibodies to *Chlamydia trachomatis* in infertility due to distal tubal disease. *Lancet* 1982; ii:574–7.
74. Moritz E. Wurmvorsatzveränderungen nach Tubenentzündungen. *Z Geburtsh Gynkol* 1912; 70: 404–16.
75. Moulder JW. Intracellular parasitism: life in an extreme environment. *J Infect Dis* 1974; 130:300–6.
76. Müller-Schoop JW, Wang S-P, Münzinger J, Schläpfer HU, Knoblauch M, Amman RW. *Chlamydia trachomatis* as possible cause of peritonitis and perihepatitis in young women. *Br Med J* 1978; i:1022–4.
77. Nordwall S. An instrument to aid laparoscopic diagnosis. *Acta Obstet Gynecol Scand* 1970; 48:111–12.
78. O'Neill MB, Moore DB. Periappendicitis: Clinical reality or pathologic curiosity. *Am J Surg* 1977; 134: 356–7.
79. Onsrud M. Perihepatitis in pelvic inflammatory disease. Association with intrauterine contraception. *Acta Obstet Gynecol Scand* 1980; 59:69–71.
80. Osser S, Persson K. Postabortal pelvic infection associated with *Chlamydia trachomatis* and the influence of humoral immunity. *Am J Obstet Gynecol* 1984; 150:699–703.
81. Paavonen J, Saikku P, Vesterinen E, Aho K. *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979; 55:203–6.
82. Paavonen J, Saikku P, von Knorring J, Aho K, Wang S-P. Association of infection with *Chlamydia trachomatis* with Fitz-Hugh-Curtis syndrome. *J Infect Dis* 1981; 144:176.
83. Paavonen J, Vesterinen E, Meyer B, Saksela E. Colposcopic and histologic findings in cervical chlamydial infection. *Obstet Gynecol* 1982; 59:712–15.
84. Paavonen J, Vesterinen E. *Chlamydia trachomatis* in cervicitis and urethritis in women. *Scand J Infect Dis* 1982; 32(Suppl):45–53.
85. Paavonen J, Kiviat N, Brunham R, et al. Prevalence and manifestations of endometritis among women with cervicitis. *Am J Obstet Gynecol* 1985; 151. In print.
86. Paavonen J, Aine R, Teisala K, Heinonen PK, Punnonen R. Comparison of endometrial biopsy and peritoneal fluid cytologic testing with laparoscopy in the diagnosis of acute pelvic inflammatory disease. *Am J Obstet Gynecol* 1985; 151:645–50.
87. Paavonen J, Aine R, Heinonen PK, et al. Chlamydial endometritis. *J Clin Pathol*, in print.
88. Paavonen T, Andersson LC, Adlercreutz H. Sex hormone regulation of *in vitro* immune response. Estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures. *J Exp Med* 1981; 154:1935–45.
89. Patton DL, Halbert SA, Kuo CC, Wang S-P, Holmes KK. Repeated chlamydial genital tract infection in pig-tailed monkeys — induction of distal tubal obstruction. Submitted to the 6th International Meeting of the International Society for STD Research. Brighton, England, July 1985.
90. Poynard T, Mazon MC, Vacherot B, et al. Périhépatite a *Chlamydia trachomatis* étude de cinq cas et revue de la littérature. *Gastroenterol. Clin Biol* 1982; 6:321–5.
91. Quigstad E, Skaug K, Jerve F, Fylling P, Ulstrup JC. Pelvic inflammatory disease associated with *Chlamydia trachomatis* infection after therapeutic abortion. *Br J Vener Dis* 1983; 59:189–92.
92. Rees E, Tait IA, Hobson D, et al. Chlamydia in relation to cervical infection and pelvic inflammatory disease. In: Nongonococcal Urethritis and Related Infections. KK Holmes, D Hobson, eds. Washington, D.C., American Society for Microbiology 1977; pp 67–76.
93. Reichert JA, Valle RF. Fitz-Hugh-Curtis Syndrome: A laparoscopic approach. *JAMA* 1976; 236:266–8.
94. Reimann K, Lind I. An indirect haemagglutination test for demonstration of gonococcal antibodies using gonococcal pili as antigen. *Acta Path Microbiol Scand* 1977; 85:115–22.
95. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977; 6:328–31.
96. Ripa KT, Svensson L, Mårdh P-A, Weström L. *Chlamydia trachomatis* cervicitis in gynecologic outpatients. *Obstet Gynecol* 1978; 52:698–702.
97. Schachter J, Caldwell HD. Chlamydiae. *Ann Rev Microbiol* 1980; 34:285–309.
98. Schildt BE, Eisemann B. Peritoneal absorption of Cr⁵¹-tagged erythrocytes. Its influence by pneumoperitoneum and Fowler's position. *Acta Clin Scan* 1960; 119:397–410.
99. Schlievert P, Larsen B, Johnson W, Galask RP. Bacterial growth inhibition by amniotic fluid. III. Demonstration of the variability of bacterial growth inhibition by amniotic fluid with a new plate-count technique. *Am J Obstet Gynecol* 1975; 122:809–13.

100. Schlievert P, Johnson W, Galask RP. Bacterial growth inhibition by amniotic fluid. VI. Evidence for a zinc-peptide antibacterial system. *Am J Obstet Gynecol* 1976; 125:906–10.
101. Schlievert P, Johnson W, Galask RP. Bacterial growth inhibition by amniotic fluid. VII. The effect of zinc supplementation on bacterial inhibitory activity of amniotic fluids from gestation of 20 weeks. *Am J Obstet Gynecol* 1977; 127:603–8.
102. Semchysyn S. Fitz-Hugh and Curtis syndrome. *J Reprod Med* 1979; 22:45–8.
103. Stajano C. La reaccion frenica en ginecologia. *Semana Med* 1920; 27:243–8.
104. Stanley MM. Gonococcal peritonitis of the upper part of the abdomen in young women. *Arch Intern Med* 1946; 78:1–13.
105. Stirling P, Richmond S. The developmental cycle of *Chlamydia trachomatis* in McCoy cells treated with cytochalasin. *J Gen Microbiol* 1977; 100:31–42.
106. Sundal E, Stalder GA. Perihepatitis gonorrhoea. Fitz-Hugh-Curtis-Syndrome. *Schweiz Med Wschr* 1980; 110:540–3.
107. Svensson L, Weström L, Ripa KT, Mårdh P-A. Differences in some clinical and laboratory parameters in acute salpingitis related to culture and serologic findings. *Am J Obstet Gynecol* 1980; 138:1017–21.
108. Svensson L, Weström L, Mårdh P-A. *Chlamydia trachomatis* in women attending a gynecological outpatient clinic with lower genital tract infection. *Br J Vener Dis* 1981; 57:259–62.
109. Svensson L, Mårdh P-A, Weström L. Infertility after acute salpingitis — with special reference to *Chlamydia trachomatis* associated infections. *Fertil Steril* 1983; 40:322–9.
110. Svensson L, Weström L, Mårdh P-A. Contraceptives and acute salpingitis. *JAMA* 1984; 251:2553–5.
111. Svensson L. Chlamydial salpingitis. Dissertation, Lund, Sweden, Studentlitteratur, 1983.
112. Sweet RL, Draper DL, Schachter J, James J, Hadley WK, Brooks GF. Microbiology and pathogenesis of acute salpingitis as determined by laparoscopy: what is the appropriate site to sample. *Am J Obstet Gynecol* 1980; 138:985–9.
113. Sweet RL. Chlamydial salpingitis and infertility. *Fertil Steril* 1982; 38: 530–2. Editorial.
114. Sweet RL, Yonekura ML, Hill G, Gibbs RS, Eschenbach DA. Appropriate use of antibiotics in serious obstetric and gynecologic infections. *Am J Obstet Gynecol* 1983; 146:719–39.
115. Terho P. *Chlamydia trachomatis* in nonspecific urethritis. *Br J Vener Dis* 1978; 54:251–6.
116. Thadepalli H, Appleman M, Maidman JE, Arce JJ, Davidson EC. Antimicrobial effect of amniotic fluid against anaerobic bacteria. *Am J Obstet Gynecol* 1977; 127:250–4.
117. Thompson SE, Hager WD, Wong K-H, et al. The microbiology and therapy of acute pelvic inflammatory disease in hospitalized patients. *Am J Obstet Gynecol* 1980; 136:179–86.
118. Thornell E, Malmgren S. Genital chlamydia-infektion orsakade akut perappendicit och akut perikolit. *Läkartidningen* 1985; 82:857.
119. Toth A, O'Leary WM, Ledger W. Evidence for microbial transfer by spermatozoa. *Obstet Gynecol* 1982; 59:556–9.
120. Trehan JD, Darougar S, Jones BR. Modification of the microimmuno-fluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977; 30:510–17.
121. Trimble C. Gonococcal perihepatitis simulating acute cholecystitis. *Surg Gynecol Obstet* 1970; 130:54–6.
122. Van Beers D, Liesnard C, Bourgeois N, Hubrechts JM. *Chlamydia trachomatis* perihepatitis. *Acta Clin B* 1982; 37:323–4.
123. von Knorring J and Nieminen J. Gonococcal perihepatitis in a surgical ward. *Ann Clin Res* 1979; 11: 66–70.
124. Wager GP, Martin DH, Koutsky L, Eschenbach DA, et al. Puerperal infectious morbidity: Relationship to route of delivery and to antepartum *Chlamydia trachomatis* infection. *Am J Obstet Gynecol* 1980; 138: 1028–33.
125. Wang S-P, Grayston JT. Local and systemic antibody response to trachoma eye infection in monkeys. In: Nichols RL, eds. *Trachoma and related disorders caused by chlamydial agents*. Amsterdam, Excerpta Medica, 1971.
126. Wang S-P, Grayston JT. Classification of TRIC and related strains with microimmunofluorescence. In: Nichols RL, eds. *Trachoma and related disorders caused by chlamydial agents*. Amsterdam, Excerpta Medica, 1971, pp 305–21.
127. Wang S-P, Eschenbach DA, Holmes KK, Wager G, Grayston JT. *Chlamydia trachomatis* infection in Fitz-Hugh-Curtis syndrome. *Am J Obstet Gynecol* 1980; 138:1034–8.
128. Wasserheit JN, Bell TA, Kiviat NB, Wølner-Hanssen P, et al. Microbiologic findings and efficacy of clindamycin plus tobramycin in laparoscopically and histologically verified pelvic inflammatory disease. Submitted.
129. Weström L. Effect of acute pelvic inflammatory disease on fertility. *Am J Obstet Gynecol* 1975; 121: 707–13.
130. Weström L, Bengtsson L-P, Mårdh P-A. The risk of pelvic inflammatory disease in women using intrauterine contraceptive devices as compared to nonusers. *Lancet* 1976; 2:221–4.
131. Weström L, Iosif S, Svensson L, Mårdh P-A. Infertility after acute salpingitis: Results of treatment with different antibiotics. *Curr Ther Res* 1979; 6(Suppl): 752–9.
132. Weström L. Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. *Am J Obstet Gynecol* 1980; 138:880–2.
133. Weström L, Svensson L, Wølner-Hanssen P, Mårdh P-A. Chlamydial and gonococcal infections in a defined population of women. *Scand J Infect Dis* 1982; 32(Suppl):157–62.
134. Wølner-Hanssen P, Weström L, Mårdh P-A. Chlamydial perihepatitis. *Scand J Infect Dis* 1982; 32 (Suppl):77–82.

135. Wølner-Hanssen P. Can oral contraceptive use modify the manifestations of pelvic inflammatory diseases? Submitted.
136. Wood JJ, Bolton JP, Cannon SR, Allan A, O'Connor BH, Darougar S. Biliary-type pain as a manifestation of genital tract infection: the Curtis-Fitz-Hugh syndrome. *Br J Surg* 1982; 69:251–3.
137. Young E, Taylor HR. Immune mechanisms in chlamydial eye infection: cellular immune responses in chronic and acute disease. *J Infect Dis* 1984; 150: 745–51.

APPENDIX: PUBLICATIONS I – IX

Reprinted by courtesy of the American Venereal Disease Association

Endometrial Infection in Women with Chlamydial Salpingitis

PÅL WØLNER-HANSEN, MD, PER-ANDERS MÅRDH, MD, BIRGER MØLLER, MD, AND LARS WESTRÖM, MD

Endometrial contents collected by a protected aspiration method from 18 women with acute salpingitis were studied by culture. *Chlamydia trachomatis* was recovered from the endometrial samples of eight women and from the cervix of six of them. *C. trachomatis* was isolated from the cervix, but not from the endometrium, of seven women. Twelve of the 15 patients who harbored *C. trachomatis* in the genital tract developed a significant antibody response to the organism. The study suggests that *C. trachomatis* spreads canalicularly from the cervix to the fallopian tubes and that this organism can cause endometrial infection.

CHLAMYDIA TRACHOMATIS has been associated with cervicitis¹ and salpingitis.²⁻⁵ Perihepatitis is another complication that can result from chlamydial infection of the female genital tract.⁶

Salpingitis, whether caused by *C. trachomatis*⁷ or *Neisseria gonorrhoeae*,⁸ is histologically similar. In gonococcal salpingitis, the infection reaches the fallopian tubes from the cervix via the endometrium.⁸ Studies on experimental salpingitis in monkeys³ indicated that *C. trachomatis*, like *N. gonorrhoeae*, spreads canalicularly to the tubes.

In this study, we present evidence showing that endometritis in women is a manifestation of canalicular spread of genital infection with *C. trachomatis*.

Patients and Methods

The study comprised 18 of 132 women consulting the Department of Obstetrics and Gynecology at the University Hospital, Lund, Sweden, during February–October 1980, who had a probable diagnosis of acute salpingitis. All 18 women had laparoscopic signs of acute salpingitis but had no previous history of acute salpingitis. They had not taken any antibiotics for three weeks before admission, and all had given a witnessed consent for endometrial aspiration. Moreover, only those pa-

From the Department of Obstetrics and Gynecology, University Hospital, Lund, Sweden; the Institute of Medical Microbiology, University of Lund, Lund, Sweden; and the Department of Obstetrics and Gynecology, County Hospital, Århus, Denmark

tients were included who could be operated on in the daytime by one of the authors. The ages of the 18 women ranged from 17 to 37 years (mean, 23 years). Two patients were classified as having severe, four as having moderately severe, and 12 as having mild inflammatory changes of the fallopian tubes.

The laparoscopic technique and the criteria of a laparoscopic diagnosis of acute salpingitis have been described in detail elsewhere.⁹

Specimens for the isolation of *C. trachomatis*, *N. gonorrhoeae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* were collected by rotation of swabs in the cervical canal and the urethra after exposure of the cervical os and the urethral orifice by insertion of a sterile Kristeller's speculum and a depressor into the vagina. Rectal specimens were also examined for *N. gonorrhoeae*. The specimens for culture of *C. trachomatis* were collected by use of cotton-tipped aluminium sticks, and those for culture of *M. hominis* and *U. urealyticum* by use of cotton-tipped wooden sticks. Specimens for isolation of *N. gonorrhoeae* were collected with wooden sticks tipped with charcoal-treated cotton.

After the specimens were obtained, the patients were hospitalized. On the next morning, they were examined by a senior gynecologist. From those patients who fulfilled the criteria for inclusion in the study (except for the laparoscopic diagnosis), new specimens were collected for culture studies in the same way as described above. The patients were then prepared for surgery.

Endometrial contents were collected transcervically. In the operating room the vagina and cervical os were cleansed with 0.1% chlorhexidine solution. Next, a plastic tube, fitted with a mandrin (Pro-Ception Fertility Cannula®, Millex Products, Inc., Chicago, Ill.), was introduced through the cervical canal. After the mandrin was replaced with a 25-cm long baby-feeding tube that

This study was supported by a grant from the World Health Organization and by grant no. 16X-4509 from the Swedish Medical Research Council.

We thank Camilla Segerström and Eva Sandgren for excellent technical assistance.

Reprint requests: Pål Wølner-Hansen, MD, Department of Obstetrics and Gynecology, University Hospital, S-221 85 Lund, Sweden.

Received for publication on September 21, 1981, and in revised form on November 16, 1981.

TABLE 1. Results of Cultural, Histologic, and Serologic Studies of 18 Patients with Acute Salpingitis

Patient No.	Cultural Results*			Histologic Signs of Endometritis	Titer of Microimmunofluorescent IgG Antibody to <i>C. trachomatis</i>	
	Cervix	Endometrium	Fallopian Tubes		Acute	Convalescent
1	C, U	C	0	NE	128	512
2	C, M, U	C, U	0	+	128	512
3	C, M, U	C	0	NE	16	256
4	C, G, U	C, G	0	-	16	256
5	C, M, U	C	0	+	64	512
6	0	C, U	C	NE	512	4,096
7	M, U	C	0	NE	256	1,024
8	C, M	C	0	NE	128	256
9	C	0	NE	NE	1,024	256
10	C	0	0	NE	512	8,192
11	C, G, M	0	C	+	32	256
12	C, U	0	0	-	4,096	8,192 (512)†
13	C, G, M, U	0	0	+	<8	32
14	C, G, M, U	0	0	NE	128	64
15	C	0	0	NE	16	32
16	G, U	G	0	-	<8	<8
17	M	0	0	NE	<8	<8
18	0	0	0	-	<8	<8

* Abbreviations: C = *Chlamydia trachomatis*; U = *Ureaplasma urealyticum*; M = *Mycoplasma hominis*; G = *Neisseria gonorrhoeae*; 0 = no organisms recovered; NE = not examined.

† Titer at 16 weeks after admission.

was adapted to a syringe, the contents of the uterine cavity were aspirated. Sampling sticks of the type mentioned above were soaked with the aspirated specimens for culture of microorganisms. Subsequently, eight of the 18 patients had a curettage performed for histologic examination of endometrial biopsies.

At laparoscopy minute biopsy specimens were collected from the fimbriae of the fallopian tubes of 16 of the 18 patients. After the specimens were crushed in 0.9% NaCl, sampling sticks, again of the kind mentioned above, were soaked with the mixture for culturing. Specimens for cultures were collected from the fallopian tubes of one patient (no. 11) by needle aspiration because the abdominal orifices were closed.

The culture methods for *C. trachomatis*,¹⁰ *N. gonorrhoeae*,¹¹ *M. hominis*,¹² and *U. urealyticum*¹² have been described elsewhere. Antibiotic treatment was instituted after completion of the surgical procedure.

Results

Table 1 shows the results of the culture studies. *C. trachomatis* was isolated from the cervix of 13 of the 18 patients and also from the endometrial aspirates from six of these 13. In two cases (no. 6 and 7), the endometrial, but not the cervical cultures, yielded growth of chlamydiae. *C. trachomatis* also was recovered from the fallopian tubes of two patients (no. 6 and 11). *N. gonorrhoeae* was isolated from the cervix of five patients, two of whom had the organism in endometrial samples also. *M. hominis* was recovered from

the cervix of nine of the 18, but never from endometrial contents. *U. urealyticum* was recovered from the cervix of ten patients and from the endometrial contents of one of the 10. Endometrial, but not cervical, cultures of patient no. 6 yielded growth of ureaplasmas. Neither *M. hominis*, *N. gonorrhoeae*, nor *U. urealyticum* was found in tubal specimens of any patient.

Twelve of the 18 patients had a fourfold or greater change in titer of IgG antibody to *C. trachomatis*; all 12 harbored the organism in the genital tract.

Histologic examination revealed a marked inflammation of the endometrium in four of the eight patients from whom endometrial biopsies were obtained. The endometrium of these patients was infiltrated with plasma cells and polymorphonuclear leukocytes (figs. 1 and 2). The endometrial cultures of two of the four women with histologic signs suggestive of endometritis yielded growth of *C. trachomatis*.

Metrorrhagic bleedings before admission were reported by eight of the 18 patients. Among the eight whose endometrial contents yielded *C. trachomatis*, five (no. 1-3, 5, and 6) had experienced metrorrhagic bleedings, whereas three (no. 9, 13, and 14) of the endometrial culture-negative patients reported similar complaints.

Discussion

Fifteen of the 18 patients with salpingitis whom we studied had serologic and/or cultural evidence of chlamydial infection. Ten of the 15 also had cultural and/

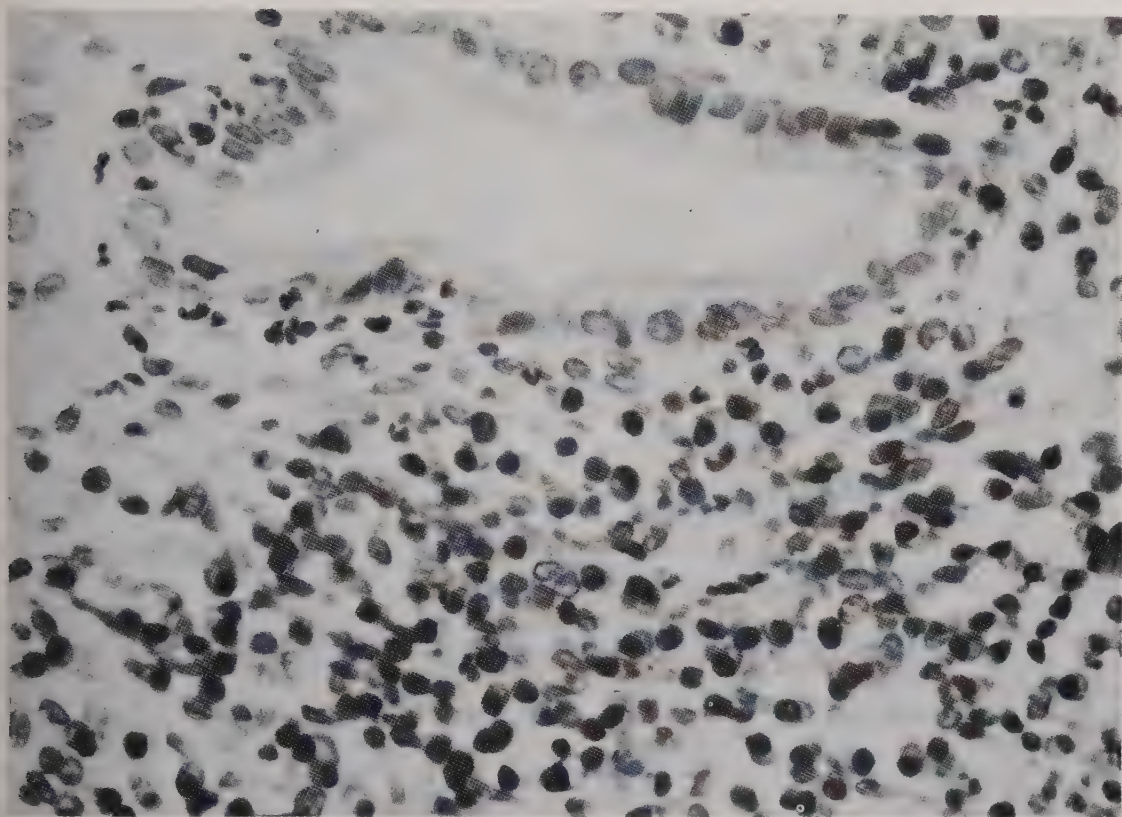


Fig. 1. Endometrial biopsies specimen from a woman with acute salpingitis and histologic signs of endometritis. The endometrium is infiltrated with plasma cells.

or histologic signs suggestive of endometritis, i.e., growth of *C. trachomatis* in the uterine mucosa and/or infiltration of the latter with plasma cells.

Difficulties are encountered in obtaining selective endometrial cultures. Previous studies of samples collected by various transcervical methods revealed that bacteria can be isolated from the endometrium of the apparently healthy woman.^{14,15} However, cultures of transfundally collected endometrial specimens obtained during hysterectomy demonstrated that the uterine cavity is usually sterile.^{15,16} Thus, it appeared that endometrial specimens obtained by the transcervical route might be contaminated with cervical microorganisms. Cervicitis is almost always present in patients with acute salpingitis; this factor makes contamination by the cervical flora even more probable. Knuppel et al.¹⁷ achieved a marked reduction in such contamination by obtaining transcervical specimens with telescoping Teflon® catheters housing a nylon bristle brush on a retractable wire in the inner cannula. Ledger et al.¹⁸ avoided cervical

contamination by performing transabdominal endometrial aspiration in women with postpartum pelvic infections. However, in menstruating women this approach would be extremely difficult, even under laparoscopic control, since the uterine cavity is just a narrow slit.

In the present study, *M. hominis* was recovered from cervical specimens of nine patients. However, this microorganism was not recovered from any endometrial aspirates. *N. gonorrhoeae* was recovered from cervical and endometrial specimens of five and two women, respectively, and *U. urealyticum*, from cervical and endometrial specimens of ten and two women, respectively. These figures seem to suggest that the device employed for endometrial sampling by the present authors possessed some selectivity for the organisms studied, although contamination in single instances can never be excluded. Moreover, two patients had cervical cultures negative for *C. trachomatis*, although cervical specimens had been collected twice, once on each of the two successive days. Both women had *C. trachomatis*

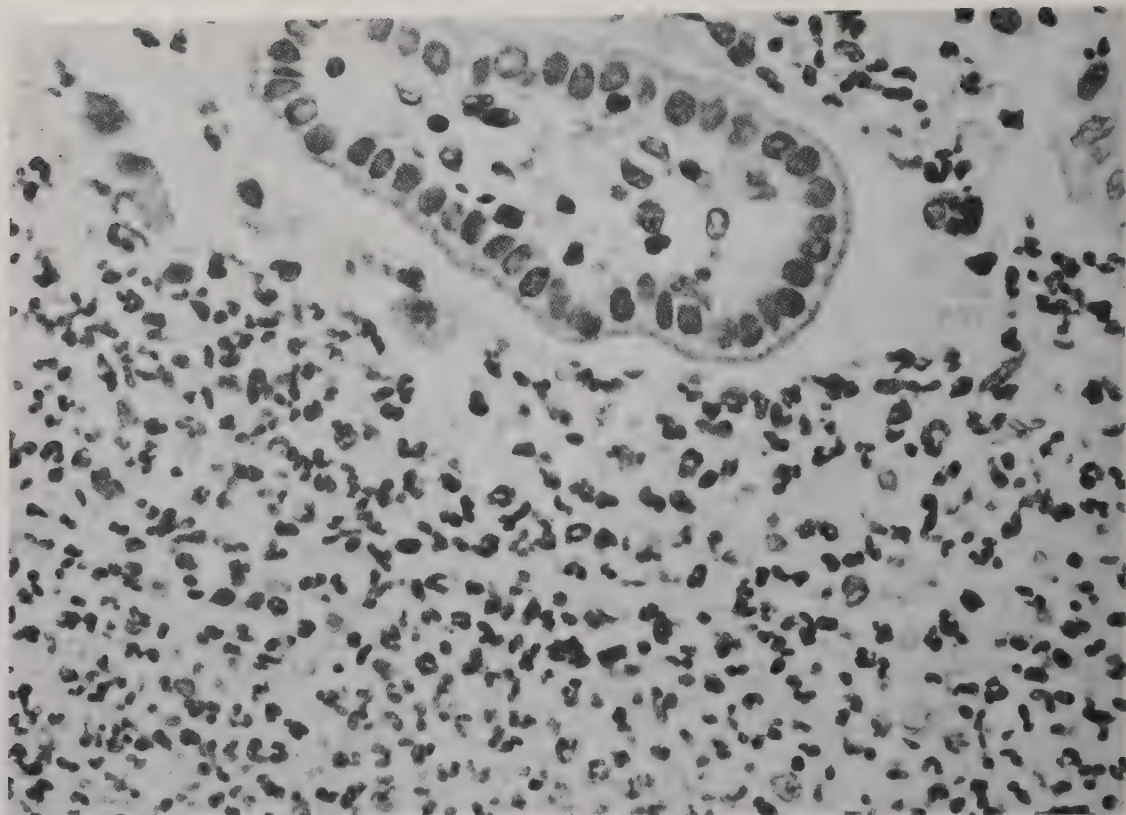


Fig. 2. Endometrial biopsy specimen from a woman with acute salpingitis and histologic signs of endometritis. Note infiltration of the endometrium by polymorphonuclear leukocytes.

in the endometrial aspirates. Thus, contamination seems improbable in these two cases. Moreover, it may be concluded that a cervical culture negative for *C. trachomatis* does not exclude a chlamydial infection in patients with acute salpingitis.

The histologic diagnosis of acute endometritis is difficult to establish. Just before menstrual desquamation and bleeding, there is a massive infiltration of the endometrium by leukocytes, mainly polymorphonuclear cells, but also macrophages. Although this cell infiltration is a normal process¹⁹ it might be mistaken for endometritis. Follicular collections of lymphocytes in the endometrium are observed frequently in premenopausal women.²⁰ Accordingly, endometritis can be overdiagnosed. On the other hand, a patchy localization of inflammatory changes in the endometrium can lead to failure to diagnose endometritis by histologic methods.⁸

Patients no. 2 and 5 had a marked infiltration of plasma cells and polymorphonuclear leukocytes; in pa-

tients no. 11 and 13 the cell infiltration was less dense. Three of these four patients had irregular uterine bleeding, and the fourth patient used oral contraceptives. Thus, none of these four patients were in the premenstrual phase of a normal physiologic cycle.

Neisseria gonorrhoeae was isolated from the cervix of 27.8% of the patients in this study, as compared with a recovery rate of 8.3% from 60 consecutive patients with acute salpingitis who attended our clinic during 1978–1979.²¹ Whether this difference in isolation rate reflects any epidemiologic changes cannot be established by data from this series of 18 patients.

As mentioned above, the histologic alterations in the fallopian tubes of patients with chlamydial and gonococcal salpingitis are similar.^{7,8} A comparison of the findings of the present study with those of studies of gonococcal endometritis⁸ shows that this similarity seems to be true for chlamydial and gonococcal endometritis also.

Except for *C. trachomatis*, *N. gonorrhoeae* and *U.*

urealyticum were the only organisms isolated from endometrial aspirates. In grivet monkeys, ureaplasmas did not provoke salpingitis as did *C. trachomatis*²² or parametritis as did *M. hominis*.²³ Whether ureaplasmas might have contributed to the endometrial infection is not known.

All patients in this study had abdominal pain and palpatory tenderness over the adnexa. To what extent the endometrial and tubal infection itself contributed to their symptoms is unclear.

Acute onset of metrorrhagic bleeding in nonpregnant women with signs of cervicitis or salpingitis (or both) has been considered one of the major signs of endometritis. Such bleeding was reported by eight of the 18 patients in this study. No correlation was found between bleeding disturbances, on the one hand and the results of cultures or histologic studies on the other.

This study suggests that *C. trachomatis*, like *N. gonorrhoeae*, can ascend from the cervix to the uterine cavity and cause endometrial infection. The findings also suggest that endometrial infection is common in patients with acute salpingitis, since chlamydiae and/or gonococci were recovered from endometrial aspirates from half of the 18 patients. These observations also contribute to the hypothesis that both *C. trachomatis* and *N. gonorrhoeae* can spread canalicularly via the uterine mucosa to the fallopian tubes.

References

1. Tait IA, Rees E, Hobson D, Byng RE, Tweedie MCK. Chlamydial infection of the cervix in contacts of men with non-gonococcal urethritis. *Br J Vener Dis* 1980; 56:37-45.

2. Mårdh P-A, Ripa KT, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977; 296:1377-9.

3. Möller BR, Mårdh P-A. Experimental salpingitis in grivet monkeys by *Chlamydia trachomatis*. Modes of spread of infection to the Fallopian tubes. *Acta Pathol Microbiol Scand [B]* 1980; 88:107-14.

4. Paavonen J. *Chlamydia trachomatis* in acute salpingitis. *Am J Obstet Gynecol* 1980; 138:957-9.

5. Henry-Suchet J, Loffredo V. Chlamydiae and mycoplasma genital infection in salpingitis and tubal sterility. *Lancet* 1980; 1:539.

6. Wølner-Hansen P, Weström L, Mårdh P-A. Perihepatitis and chlamydial salpingitis. *Lancet* 1980; 1:901-4.

7. Möller BR, Weström L, Ahrons S, et al. *Chlamydia trachomatis* infections of the Fallopian tubes. Histological findings in two patients. *Br J Vener Dis* 1979; 55:422-8.

8. Runge H. Gonorrhoe der weiblichen Geschlechtsorgane. In: Seitz L, Amreich AT, eds. *Biologie und Pathologie des Weibes*. Vol. 5. Berlin, Innsbruck, München, Wien: Urban & Schwarzenberg, 1953:445-9.

9. Jacobson L, Weström L. Objectivized diagnosis of acute pelvic inflammatory disease: Diagnostic and prognostic value of routine laparoscopy. *Am J Obstet Gynecol* 1969; 105:1088-98.

10. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977; 6:328-31.

11. Mårdh P-A, Mårtensson D, Soltesz LV. An effective, simplified medium for the culture of *Neisseria gonorrhoeae*. *Sex Transm Dis* 1978; 5:10-13.

12. Mårdh P-A, Weström L. Tubal and cervical cultures in acute salpingitis with special reference to *Mycoplasma hominis* and T-strain mycoplasmas. *Br J Vener Dis* 1970; 46:179-86.

13. Trehanne JD, Darougar S, Jones BR. Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977; 30:510-17.

14. Willson JR, Bollinger CC, Ledger WJ. The effect of an intra-uterine contraceptive device on the bacterial flora of the endometrial cavity. *Am J Obstet Gynecol* 1964; 90:726-39.

15. Mishell DR, Bell JH, Good RG, Moyer DL. The intrauterine device: A bacteriologic study of the endometrial cavity. *Am J Obstet Gynecol* 1966; 96:119-26.

16. Ansbacher R, Boyson WA, Morris JA. Sterility of the uterine cavity. *Am J Obstet Gynecol* 1967; 99:394-6.

17. Knuppel RA, Scerbo JC, Mitchell GW, Cetulo CL, Bartlett J. Quantitative transcervical uterine cultures with a new device. *Obstet Gynecol* 1981; 57:243-8.

18. Ledger WJ, Gee CL, Pollin PA, Lewis WP, Sutter VL, Finegold SM. A new approach to patients with suspected anaerobic postpartum pelvic infections. Transabdominal uterine aspiration for culture and metronidazole for treatment. *Am J Obstet Gynecol* 1976; 126:1-6.

19. Novak ER, Woodruff JD. Novak's gynecologic and obstetric pathology. Philadelphia: WB Saunders, 1974:81-100.

20. Sen DK, Fox H. The lymphoid tissue of the endometrium. *Gynecologia* 1967; 163:371-8.

21. Mårdh P-A, Lind I, Svensson L, Weström L, Möller BR. Antibodies to *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Neisseria gonorrhoeae* in sera from patients with acute salpingitis. *Br J Vener Dis* 1981; 57:125-9.

22. Mårdh P-A, Weström L, Möller BR, Ripa KT. Pelvic inflammatory diseases. II. Clinical, etiological and pathophysiological studies. Geneva: World Health Organization working document no. INT/VDT/78.333. 1978.

23. Möller BR, Freundt EA, Mårdh P-A. Experimental pelvic inflammatory disease in grivet monkeys provoked by *Chlamydia trachomatis* and *Mycoplasma hominis*. *Am J Obstet Gynecol* 1980; 138:990-5.



PERIAPPENDICITIS AND CHLAMYDIAL SALPINGITIS

Per-Anders Mårdh, M.D., *Lund, Sweden and*

Pål Wølner-Hanssen, M.D., *Seattle, Washington*

PERIAPPENDICITIS is defined as appendiceal serositis which, unlike primary appendicitis, does not involve the intestinal mucosa (1). In a series of 224 patients who underwent appendectomy on a preoperative diagnosis of appendicitis, 12 (5.3 per cent) were found to have periappendicitis. All but one of the 12 were women (1). Gonococcal infection of the fallopian tubes might spread to the appendix (2, 3). Such secondary appendicitis was histologically demonstrated in 12 (44.4 per cent) of 27 patients with gonococcal salpingitis (3).

Chlamydia trachomatis has been shown to cause cervicitis (4), endometritis (5) and salpingitis (6). Perihepatitis has been observed to arise from chlamydial salpingitis (7, 8). The spread of the microorganism from the cervix uteri to the fallopian tubes probably is canalicular, through the uterine cavity (5). Further spread, to the hepatic region, might be paracolic, by way of the lymphatic drainage system (9). This study was done to describe seven instances of chlamydia-associated salpingitis with apparent spread of the tubal inflammation to the appendix, causing periappendicitis.

PATIENTS AND METHODS

The seven women in the study were seen during the period 1978 to 1982. They were derived from a series of 112 (6.3 per cent) women who were treated at this gynecology department for laparoscopically verified salpingitis with evidence of *Chlamydia trachomatis* but not *Neisseria gonorrhoeae* infection.

Specimens for isolation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were collected from the cervical canal and urethra as previously described (10). Rectal specimens were studied for gonococci. In one of seven patients with periappendicitis, samples were taken from the uterine cavity with a protected transcervical aspiration method and from the fallopian tubes (10).

From the Department of Medical Microbiology, University of Lund, and the Department of Obstetrics and Gynecology, University Hospital, Lund.

Reprint requests: Dr. P. Wølner-Hanssen, Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, Washington 98195.

The techniques used for isolation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were as previously described (11, 12). A modified microimmunofluorescence test was used to detect serum IgM and IgG antibodies to *Chlamydia trachomatis* (13). The laparoscopic technique and the criteria for grading the inflammatory changes in the fallopian tubes were in accordance with earlier recommendations (14).

Appendectomy was performed in the seven women because primary appendicitis could not be excluded at the laparoscopic examination performed for salpingitis. The appendices were examined histologically after fixation in 20 per cent formaldehyde and staining with eosin and hematoxylin.

Sections of the appendix were stained with fluorescein labeled monoclonal antibodies to *Chlamydia trachomatis* (MikroTrak, *Chlamydia trachomatis* Culture Confirmation Test, Syva Co.). Prior to staining, the formaldehyde fixed, wax embedded preparations were treated with xylol, 99 and 96 per cent, and deionized water in order to remove the wax. The appendiceal sections were then overlaid with monoclonal antibodies and incubated for 30 minutes at 37 degrees C. in a moist chamber. The specimens were rinsed in phosphate-buffered saline solution (pH 7.2) for ten minutes and in deionized water for ten seconds. After air drying, the preparations were mounted with glycerol and studied under a Leitz fluorescence microscope.

RESULTS

Some clinical manifestations and laboratory findings in the seven patients are shown in Tables I and II. The inflammatory changes revealed by laparoscopic examination were milder in the left than in the right oviduct in three of the seven women, and in two of the other patients, only the right tube was inflamed. In six of the seven patients, the appendix was adherent to the right adnexa.

Cultures for *Chlamydia trachomatis* were made from the cervix uteri only in six of the seven women, five of whom harbored the organism at

TABLE I.—CLINICAL SYMPTOMS OF PATIENTS WITH PERIAPPENDICITIS AND SALPINGITIS

Patient No.	Lower abdominal pain, right/left	Adnexal tenderness, right/left	Purulent vaginal discharge	Abnormal bleeding
1	+/+	+/+	+	0
2	0/+	+/0	?*	+
3	+/+	+/+	+	+
4	+/0	+/0	+	+
5	+/+	+/+	+	0
6	+/+	+/+	+	+
7	+/0	+/+	+	+

*Uterine bleeding.

TABLE II.—LABORATORY FINDINGS OF PATIENTS WITH PERIAPPENDICITIS AND SALPINGITIS

Patient No.	ESR	WBC	Rectal temperature	Antibody to <i>Chlamydia trachomatis</i> *—	
				Acute	Convalescent
1	28	18.1	38.1	N.D.	32
2	34	8.4	37.7	256	256
3	62	8.3	37.6	512	N.D.
4	28	8.9	37.8	<8	64
5	30	8.4	37.8	128	512
6	90	10.3	37.5	1,024	4,096
7	35	9.5	36.7	512	N.D.

*Microimmunofluorescent serum IgG antibody titer.

ESR, Erythrocyte sedimentation rate.

WBC, White blood cell count ($\times 10^6$ /liter).

this site. One patient (Patient 7) had been culture-positive for *Chlamydia trachomatis* in the cervix uteri ten days before admission to this department. In the interval, she had received no treatment, but a repeat cervical culture yielded no growth of the organism. In the remaining patient (Patient 6), cultures were made from the cervix uteri, endometrium and fallopian tubes. *Chlamydia trachomatis* was isolated only from the last two sites. *Neisseria gonorrhoeae* was not isolated from any patient.

More than one serum sample was obtained from four patients (Table II), in three of whom there was a fourfold or greater increase in the titer of IgG antibody to *Chlamydia trachomatis*. In the fourth patient, the titer was 256 in two samples with an interval of one month. In two of the three women with only one serum sample, the IgG antibody titer was 512. One of these two patients (Patient 3) also had IgM antibodies, in a titer of 16.

The results of histologic study of all seven appendices showed periappendicitis. The mucosal layer was intact, but there was leukocytic infiltration of the serosa and subserosa. In the sections studied with immunofluorescence, no chlamydial inclusions were revealed.

DISCUSSION

The results of the present study demonstrated that periappendicitis may be secondary to chlamydia associated salpingitis. Among the 112 consecutive patients who were chlamydia positive with acute salpingitis, seven (6.3 per cent) also had histologically confirmed periappendicitis. This prevalence of periappendicitis in patients with salpingitis is in accordance with that of previous reports (1, 15).

All seven patients had been admitted to a department of gynecology because of symptoms suggesting acute salpingitis (16). Thus, five of the even had bilateral pain and adnexal tenderness. The erythrocyte sedimentation rate was fairly

high in all patients, whereas only one patient had elevated white blood cell count or rectal temperature higher than 38 degrees C. The appendiceal infection was an unexpected finding at laparoscopic examination. Because primary appendicitis could not be excluded at this examination, the appendix was resected in all of the patients.

Primary and secondary appendicitis may not be clinically distinguishable from acute salpingitis (15). Periappendicitis is not an uncommonly encountered entity in surgical departments. In a study of 70 women with acute appendicitis, the condition was of secondary type in 11 (15.2 per cent) (1). Seven of the 11 patients had other conditions affecting the tubes or ovaries. The nature of these conditions was not specified, however. In another study (17), seven instances (4.2 per cent) of periappendicitis were reported among 167 patients who had undergone appendectomy and in whom appendicitis was histologically verified.

All seven patients in the present study were infected with *Chlamydia trachomatis*. An association between this microorganism and the acute disease was proved by culture or serologic studies, or both, in four of the seven patients (Patients 3, 4, 5 and 6). Regrettably, no cultures were made from the resected appendices. The results of studies of appendiceal sections that were stained with monoclonal antibodies to *Chlamydia trachomatis* revealed no chlamydial inclusions. Thus, a chlamydial cause of the periappendicitis was not proved in this study but seemed probable in four.

Patients with primary appendicitis usually receive no antibiotic treatment postoperatively. For periappendicitis secondary to salpingitis, however, such treatment seems to be mandatory. Thus, the results from the present study underline the importance of scrutinizing the pelvic organs when performing appendectomy in sexually active females assumed to have acute appendi-

cit. Postoperative antibiotic treatment of patients with periappendicitis and salpingitis should be instituted with a drug known to be active against microorganisms commonly causing acute salpingitis, that is, *Chlamydia trachomatis*, *Mycoplasma hominis* and *Neisseria gonorrhoeae*. *Chlamydia trachomatis* seems currently to be the most common cause of acute salpingitis, at any rate, in northern Europe (18). The epidemiologic measures in women with acute salpingitis and periappendicitis should include examination and treatment of the sexual partners of the patients.

SUMMARY

Periappendicitis seems to be a novel manifestation of infections with *Chlamydia trachomatis*. In seven of 112 women with laparoscopically verified acute salpingitis, secondary appendicitis was diagnosed and histologically confirmed. The genital tract in all seven patients harbored *Chlamydia trachomatis* but not *Neisseria gonorrhoeae*. The fallopian tubes should be scrutinized when an inflamed appendix is removed from a sexually active woman. Signs of salpingitis should then lead to appropriate microbiologic, therapeutic and epidemiologic measures, including contact tracing.

REFERENCES

1. O'NEILL, M. B., and MOORE, D. B. Periappendicitis: Clinical reality or pathologic curiosity. *Am. J. Surg.*, 1977, 134: 356-357.
2. AUMAN, G. L., and WALDENBERG, L. M. Gonococcal periappendicitis and salpingitis in a prepubertal girl. *Pediatrics*, 1976, 58: 287-288.
3. MORITZ, E. Wurmvorsatzveränderungen nach Tuberculentzündungen. *Z. Geburtsh. Gynkol.*, 1912, 70: 404-416.
4. DUNLOP, E. M. C., HARPER, J. A., AL-HUSSAINI, M. K., and others. Relation of TRIC agent to "nonspecific"

- genital infection. *Br. J. Vener. Dis.*, 1966, 42: 77-87.
5. MÅRDH, P.-A., MØLLER, B. R., INGERSLEV, H. J., and others. Endometritis caused by *Chlamydia trachomatis*. *Br. J. Vener. Dis.*, 1981, 57: 191-195.
6. MÅRDH, P.-A., RIPA, K. T., SVENSSON, L., and WESTRÖM, L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N. Engl. J. Med.*, 1977, 296: 1377-1379.
7. WØLNER-HANSEN, P., WESTRÖM, L., and MÅRDH, P.-A. Perihepatitis and chlamydial salpingitis. *Lancet*, 1980, 1: 901-904.
8. WØLNER-HANSEN, P., SVENSSON, L., WESTRÖM, L., and MÅRDH, P.-A. Isolation of *Chlamydia trachomatis* from the liver capsule in Fitz-Hugh-Curtis syndrome. *N. Engl. J. Med.*, 1982, 306: 113.
9. WØLNER-HANSEN, P., WESTRÖM, L., and MÅRDH, P.-A. Chlamydial perihepatitis. *Scand. J. Inf. Dis.*, 1982, Suppl. 32: 77-82.
10. MÅRDH, P.-A., WESTRÖM, L., COLLEEN, S., and WØLNER-HANSEN, P. Sampling, specimen handling, and isolation techniques in the diagnosis of chlamydial and other genital infections. *Sex. Transm. Dis.*, 1981, 8: 280-285.
11. MÅRDH, P.-A., MÅRTENSSON, D., and SOLTESZ, L. V. An effective, simplified medium for the culture of *Neisseria gonorrhoeae*. *Sex. Transm. Dis.*, 1978, 5: 10-13.
12. RIPA, K. T., and MÅRDH, P.-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J. Clin. Microbiol.*, 1977, 6: 328-331.
13. TREHARNE, J. D., DAROUGAR, S., and JONES, B. R. Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J. Clin. Pathol.*, 1977, 30: 510-517.
14. JACOBSSON, L., and WESTRÖM, L. Objectivized diagnosis of acute pelvic inflammatory disease. *Am. J. Obstet. Gynecol.*, 1968, 105: 1088-1098.
15. SAMUELSSON, S. Differential diagnosis of pelvic appendicitis and acute conditions of uterine appendages. *Acta Obstet. Gynecol. Scand.*, 1957, Suppl. 9, 26: 5-28.
16. WØLNER-HANSEN, P., MÅRDH, P.-A., SVENSSON, L., and WESTRÖM, L. Laparoscopy in women with chlamydial infection and pelvic pain. *Obstet. Gynecol.*, 1983, 61: 299-303.
17. BUTLER, C. Surgical pathology of acute salpingitis. *Human Pathol.*, 1981, 12: 870-878.
18. MÅRDH, P.-A., MØLLER, B. R., and PAAVONEN, J. Chlamydial infection of the female genital tract with emphasis on pelvic inflammatory disease. *Sex. Transm. Dis.*, 1981, 8: 140-155.

PERIHEPATITIS AND CHLAMYDIAL SALPINGITIS

P. WÖLNER-HANSEN

L. WESTRÖM

P. -A. MÅRDH

*Department of Obstetrics and Gynaecology, University
Hospital, Lund; and Institute of Medical Microbiology,
University of Lund, Lund, Sweden*

Summary In 4 patients with acute salpingitis (3 of whom also had perihepatitis) the diagnoses were verified by laparoscopy or laparotomy and all 4 had cultural and serological evidence of current infection with *Chlamydia trachomatis*, whereas none had signs of gonococcal infection. 3 of the 4 had symptoms in the right upper abdomen, but the liver surface showed signs of perihepatitis in only 2. In the 4th patient, perihepatitis was diagnosed, although she denied symptoms in the hepatic region. Thus symptoms from the right upper abdomen in a young woman may be an indirect sign of a genital infection.

Introduction

PERIHEPATITIS, as a complication of acute salpingitis, was first described by Stajano in 1919, but it did not become widely known until it was rediscovered in the 1930s.¹⁻³ Fitz-Hugh found gram-negative diplococci in his patients' cervical smears and named the condition gonococcic perihepatitis, now known as Fitz-Hugh-Curtis syndrome. *Neisseria gonorrhææ* is generally considered the cause of this condition.

Typical symptoms include pain, usually of sudden onset, in the right upper abdomen; the pain sometimes overshadows the symptoms of salpingitis. In the acute stage, there is a localised peritonitis involving the anterior liver surface and the adjacent peritoneum of the anterior abdominal wall. Later, "violin-string" adhesions may develop.⁴

Cervicitis⁵ and acute salpingitis^{6,7} may be caused by *Chlamydia trachomatis*. Recently, serological evidence has been presented of chlamydial infection in patients with peritonitis and perihepatitis, which is not associated with pelvic inflammatory disease.⁸

We describe here 3 patients with salpingitis and perihepatitis, with evidence of genital chlamydial infection. A 4th patient had salpingitis and symptoms which suggested Fitz-Hugh-Curtis syndrome, but laparoscopy

revealed a normal liver surface. No patient had evidence of gonococcal infection.

Material and Methods

All 4 patients studied were admitted to the department of obstetrics and gynaecology, at the University Hospital, Lund. One patient was also examined at the department of surgery and another at the department of paediatric surgery.

The technique of laparoscopy and the criteria for grading the inflammatory changes in the fallopian tubes are described elsewhere,⁹ as are the culture methods for *N. gonorrhoea*¹⁰ and *C. trachomatis*.¹¹ Gonococcal pili antibodies¹² and antibodies to *Mycoplasma hominis*¹³ were determined by an indirect hæmagglutination test. A modified microimmunofluorescence test¹⁴ was used for determination of IgM and IgG antibodies to *C. trachomatis*.

Case-reports

Patient 1

A 14-year-old nullipara was admitted to the department of paediatric surgery on May 3, 1979, with a 3-week history of low abdominal pain, which had moved to the right upper quadrant about 10 days previously. Physical examination revealed right upper-quadrant tenderness and liver enlargement, but there was no tenderness over the adnexa. An ultrasound scan of the gallbladder and chest X-ray were normal, but the gallbladder could not be seen on cholecystography. Laparotomy revealed violin-string adhesions between the anterior capsule of the left lobe of the liver and the anterior abdominal wall. A small abscess was found near the gallbladder. The fallopian tubes were red, swollen, and involved in adhesions. Salpingitis and perihepatitis were diagnosed.

On admission, the rectal temperature was 37.4°C, erythrocyte sedimentation rate (ESR) 67 mm/h, and the white blood-cell count (WBC) $8.8 \times 10^9/l$. The serum-amylase level had risen to 6.2 $\mu\text{kat/l}$ (normal 1.2–5.0). The serum levels for alkaline phosphatase (s-ALP), aspartate aminotransferase (s-ASAT), alanine aminotransferase (s-ALAT), and glutamyl transferase (s-GT) were normal (table I). No cultures were performed, because antibiotic therapy had already begun. The serological results are shown in table II. She had had only one sexual partner, with whom she had had regular intercourse during the 3 months before admission. They had not used any contraceptives. Shortly before admission she had had abdominal pain; and urethritis was diagnosed in her male partner. He was culture-positive for *C. trachomatis*, but gonorrhoea could not be diagnosed. She was treated with oral doxycycline (100 mg/day). After 12 days, symptoms had subsided, and she returned home. 9 days later, pelvic examination was normal.

TABLE I—RESULTS OF LABORATORY TESTS

Serum analyses	Normal values	Patient			
		1	2	3	4
Bilirubin	3–20 mmol/l	9.0	5.0	11.0	7.0
Alkaline phosphatase	0.8–4.6 μ kat/l	2.5	3.5	2.8	1.9
Aspartate aminotransferase	<0.67 μ kat/l	0.3	0.3	0.2	0.34
Alanine aminotransferase	<0.67 μ kat/l	0.2	0.3	0.2	0.18
Glutamyl transferase	<0.60 μ kat/l	0.3	0.4	0.2	0.68
Amylase	1.2–5 μ kat/l	6.2	ND	ND	2.2
ESR	<15 mm/h	67	65	44	52
WBC	4–10 $\times 10^9$ /l	8.4	11.1	5.3	ND

ND=not done.

Patient 2

An 18-year-old nullipara was admitted on Aug. 4, 1978, after 3 weeks of colicky pain in the lower abdomen. 3 days previously, she had returned to Sweden from a year in Peru. During the preceding 3 months, she had had frequent bleedings and a foul-smelling discharge. Her general condition was good. Pelvic examination revealed a tender, cystic mass in the left adnexal region and leucorrhœa. Laparoscopy showed a red-denied pelvic serosa and multiple adhesions. The fallopian tubes were red and swollen. The liver capsule had fibrinous plaques, and minute hæmorrhagic spots were seen on the serosa of the diaphragm. Moderately severe salpingitis was diagnosed. Rectal temperature was 37.2°C, ESR 65 mm/h, and WBC 11.1×10^9 /l. Serum bilirubin, s-ALP, s-ASAT, s-ALAT, and s-GT were normal (table 1). *C. trachomatis* was isolated from the cervix (table 11). She was treated with oral doxycycline (100 mg/day, by mouth). After 16 days, the symptoms had subsided and she went home. Findings were normal at follow-up examination 9 weeks later and cervical cultures showed no growth of sexually transmitted organisms.

Patient 3

A 28-year-old nullipara was admitted on Dec. 4, 1979, after 4 days of pain in the lower abdomen; she also had right upper-quadrant pain, accentuated by deep inspiration and coughing. She had slight nausea, but had not vomited. She had had normal menstrual bleeding just before the onset of pain. She had had clomiphene for infertility during the four previous menstrual cycles.

Pelvic examination revealed a blood-stained yellowish discharge, and a tender mass in the right adnexal region. Microscopical examination of the vaginal contents indicated leu-

corrhœa. Laparoscopy showed red swollen fallopian tubes. Pus appeared in the abdominal orifice of the right tube. Typical patches of endometriosis were seen on the peritoneal surface of the broad ligaments and on the anterior and posterior cul-de-sac. The liver surface and adjacent peritoneum of the anterior abdominal wall were reddened and had small white spots. The gallbladder was normal. Mildly severe salpingitis, endometriosis, and perihepatitis were diagnosed. Rectal temperature was 37.2°C, ESR 44 mm/h, and WBC $5.3 \times 10^9/l$. Serum-bilirubin, s-ALP, s-ASAT, s-ALAT, and s-GT were normal (table I). Cultures for *N. gonorrhœa*, *C. trachomatis*, and *M. hominis* from a minute specimen of the fimbriæ of the right fallopian tube were negative. Cervical cultures yielded growth of *C. trachomatis*, whereas cervical, rectal, and urethral cultures showed no growth of gonococci (table II).

She was taking part in an antibiotic double-blind study, for which the code has not yet broken. 10 days after admission her symptoms had improved, and she was discharged. 1 month later, pelvic examination revealed slight tenderness over the right salpinx, but cervical cultures yielded no growth of sexually transmitted organisms.

Patient 4

A 16-year-old nullipara was admitted on May 25, 1978, with a 2-week history of pain in the lower left quadrant of the abdomen. She had been taking oral contraceptives and had had breakthrough bleeding the previous day. Pelvic examination showed uterine bleeding and tenderness over the left adnexa. Rectal temperature was 37.2°C, and ESR was 21 mm/h. After 2 days symptoms had subsided, and she was discharged. On June 4, right upper-quadrant pain developed. 2 days later, she consulted the department of surgery; perihepatitis was suspected, and she was readmitted to the department of obstetrics and gynecology. She had tenderness over the right upper quadrant and also over the uterus and both adnexa. She had a watery uterine bleeding. Laparoscopy showed bilateral pyosalpinx with adhesions, and there was pus in the cul-de-sac. However, there were no signs of perihepatitis.

Rectal temperature was 38.2°C and ESR 52 mm/h. S-GT had risen to 0.68 $\mu\text{kat/l}$ (normal <0.60). Serum bilirubin, s-ALP, s-ASAT, and s-ALAT were within normal range (table I). Cervical cultures showed growth of *C. trachomatis* but not *N. gonorrhœa*, nor were gonococci isolated from urethral or rectal specimens (table II).

Doxycycline (100 mg/day) was given by mouth. After 12 days pelvic symptoms had subsided, and she went home. At follow-up examination 9 days later findings were normal. Cervical cultures yielded no growth of sexually transmitted organisms.

TABLE II—CULTURAL AND SEROLOGICAL RESULTS

Patient	Days after onset of abdominal pain	<i>Chlamydia trachomatis</i>			<i>Neisseria gonorrhoea</i>	
		Cervical culture	Microimmunofluorescent antibody titre		Cervical culture	Gonococcal pili antibody titre
			IgM	IgG		
1	39	ND	<8	4096	ND	<40
	48		64	2048		<40
	58		<8	2048		<40
	147		<8	256		<40
2	24	+	<8	2048	—	<40
	35		<8	2048		<40
3	4	+	<8	<16	—	<40
	14		<8	64		<40
	48		<8	32		<40
	15		<8	64		<40
4	21	+	<8	512	—	<40
	29		<8	512		<40

ND not done because antibiotic therapy had begun.

Discussion

Infection with *N. gonorrhæa* has been considered to be the cause of Fitz-Hugh-Curtis syndrome, although a search for gonococci is often negative. Litt¹⁵ isolated gonococci from the cervix of only 12 of 37 patients with perihepatitis; Semchyshyn¹⁶ found *N. gonorrhæa* in 14 of 29 such cases. There are few reports of gonococci being isolated from the peritoneal cavity.^{2,17}

C. trachomatis is at least as common an ætiological agent of sexually transmitted genital infections as *N. gonorrhæa*.¹⁸ Infections with *C. trachomatis* were the most common cause of salpingitis in the hospital catchment region to which the patients in our study belonged.⁶

Müller-Schoop et al.⁸ reported 11 young women with acute peritonitis, 9 of whom had serological evidence of a recent or current infection with *C. trachomatis*. 6 of the 9 patients had perihepatitis, but no signs of salpingitis. The other 2 patients had salpingitis, but they had no signs of perihepatitis. Thus none of the patients fulfilled the criteria for Fitz-Hugh-Curtis syndrome. No cultures for *C. trachomatis* were performed, but 3 of the patients had *N. gonorrhæa* in the cervix.

In our study patients 1, 2, and 3 had signs of salpingitis and perihepatitis, but gonorrhæa was not diagnosed. Serological studies indicated *C. trachomatis* infection in all 4 patients. *C. trachomatis* was also found in cervical specimens of 3 patients from whom cultures for this organism were performed, but it was not found in the tubal biopsy of the only patient studied this way. The inability to recover *C. trachomatis* in tubal specimens of some patients with evidence of chlamydial salpingitis (suggested by local antibody production⁷) is comparable to the difficulty in recovering *N. gonorrhæa* from the fallopian tubes in cases of gonococcal salpingitis.

The diagnoses of salpingitis and perihepatitis were confirmed by laparoscopy or laparotomy in all 3 patients. In the 14-year-old girl, biopsy showed fibrinous changes in the liver capsule, but no parenchymal changes. Thus salpingitis caused by *C. trachomatis* may be associated with perihepatitis. Such patients may (patients 1 and 3) or may not (patient 2) have symptoms and signs that suggest perihepatitis. There are also patients (e.g., patient 4) with salpingitis and symptoms that suggest of perihepatitis which is not confirmed on

visual inspection of the liver. Both perihepatitis and salpingitis,¹⁹ cannot be diagnosed accurately unless laparoscopy or laparotomy is performed.

It is possible that patients with Fitz-Hugh-Curtis syndrome consult surgical departments or hospital emergency clinics and are assumed to have pancreatitis or cholecystitis. As in patient 1, the serum-amylase values could be raised and the gallbladder invisible on cholecystography. Therefore laparoscopy should be performed in young women with right upper-quadrant pain and signs of infection in the lower genital tract. Such an infection is usually present in women with acute salpingitis.⁹ If patients with right upper-quadrant pain were tested for cervicitis the number of undiagnosed cases of salpingitis and unnecessary laparotomies might be reduced.

Liver-enzyme analyses in our patients were normal (except for 1 patient with raised s-GT). This finding indicates that chlamydia-induced perihepatitis does not affect the liver parenchyma, which is also consistent with the examination of the biopsy specimen in patient 1.

C. trachomatis infection may be even more common than gonorrhœa in salpingitis associated with perihepatitis. During the two years covered by this study no patient with gonococcal salpingitis seen by us had signs of perihepatitis.

In birds infected with *Chlamydia psittaci*, the liver may be infected and the serous membranes inflamed.²⁰ Salpingitis developed in grivet monkeys when *C. trachomatis* had been introduced into the uterine cavity after curettage.²¹ In one monkey thus infected after ligation of the fallopian tubes, salpingitis did not develop. However, perihepatitis was diagnosed at necropsy 2 weeks after infection. Culture and serological studies suggested that *C. trachomatis* was the cause. That sexually transmitted agents may spread other than by the canalicular route (through the uterine cavity and the tubes to the liver) is suggested by a report of gonococcal perihepatitis in a man with gonorrhœa.¹⁷ Also the findings of Müller-Schoop⁷ indicate that peritonitis, but not salpingitis, may be a complication of genital chlamydial infection.

This study was supported by a grant from W.H.O. and by the Swedish Medical Research Council, grant no. 16 X-4509. We thank Dr J. Lind for performing the gonococcal pili antibody tests.

REFERENCES

1. Curtis AH. A cause of adhesions in the right upper quadrant. *JAMA* 1930; **94**: 1221-22.
2. Fitz-Hugh T. Acute gonococcic peritonitis of the right upper quadrant in women. *JAMA* 1934; **102**: 2094-96.
3. Fitz-Hugh T. Acute gonococcic perihepatitis.—A new syndrome of right upper quadrant abdominal pain in young women. *Rev Gastroent* 1936; **3**: 125-31.
4. Stanley MM. Gonococcic peritonitis of the upper part of the abdomen in young women. *Arch Int Med* 1946; **78**: 1-13.
5. Rees E, Tait IA, Hobson D, Williams F, Johnson A. *Chlamydia* in relation to cervical infection and pelvic inflammatory disease. In: Hobson D, Holmes KK eds. *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society of Microbiology, 1977: 67-76.
6. Mårdh P-A, Ripa KT, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Eng J Med* 1976; **296**: 1377-79.
7. Trehan JD, Ripa KT, Mårdh P-A, Svensson L, Weström L, Darougar S. Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979; **55**: 26-29.
8. Müller-Schoop JW, Wang SP, Münzinger J, Schläpfer HU, Knoblauch M, Amman RW. *Chlamydia trachomatis* as possible cause of peritonitis and perihepatitis in young women. *Br Med J* 1978; **i**: 1022-24.
9. Weström L. Diagnosis, Aetiology, and Prognosis of Acute Salpingitis. Lund: Studentlitteratur AB, 1976: 15-17; 45.
10. Mårdh P-A, Mårtensson D, Soltesz LV. An effective, simplified medium for the culture of *Neisseria gonorrhoeae*. *Sex Transm Dis* 1978; **5**: 10-13.
11. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977; **6**: 328-31.
12. Reimann K, Lind I. An indirect haemagglutination test for demonstration of gonococcal antibodies, using gonococcal pili as antigen. I. Methodology and preliminary results. *Acta Pathol Microbiol Scand* 1977; **85**: 115-22.
13. Krogsgaard-Jensen A. Indirect hemagglutination with *Mycoplasma* antigens. Effect of pH on antigen sensitization of tanned, fresh and formalinized sheep erythrocytes. *Appl Microbiol* 1971; **22**: 756-59.
14. Trehan JD, Darougar S, Jones BR. Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977; **30**: 510-17.
15. Litt IF, Cohen ML. Perihepatitis associated with salpingitis in adolescents. *JAMA* 1978; **240**: 1253-54.
16. Semchyshyn S. Fitz-Hugh and Curtis syndrome. *J Reprod Med* 1979; **22**: 45-48.
17. Kimball MW, Knee S. Gonococcal perihepatitis in a male. *N Engl J Med* 1970; **282**: 1082-83.
18. Schachter J. Chlamydial infections. *N Engl J Med* 1978; **298**: 428-35; 490-95; 540-49.
19. Weström L, Mårdh P-A. Epidemiology, etiology, and prognosis of acute salpingitis. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society of Microbiology, 1977: 84-90.
20. Storz J. *Chlamydia* and chlamydia-induced diseases. Springfield: Charles Thomas, 1971: 136-38.
21. Möller BR, Mårdh P-A. Experimental salpingitis in grivet monkeys by *Chlamydia trachomatis*. Modes of spread of infection to the Fallopian tubes. *Acta Pathol Microbiol Scand, Sect B* (in press).

ISOLATION OF *CHLAMYDIA TRACHOMATIS* FROM THE LIVER CAPSULE IN FITZ-HUGH-CURTIS SYNDROME

To the Editor: *Chlamydia trachomatis* was isolated from the liver capsule of a patient in whom laparoscopic findings indicated acute salpingitis and perihepatitis — i.e., Fitz-Hugh-Curtis syndrome.

Perihepatitis in association with acute salpingitis was previously thought to be a complication of gonococcal infection. Reports during recent years have indicated that *C. trachomatis* may also be associated with this syndrome.¹⁻⁶ The connection of the syndrome to *C. trachomatis*, however, has hitherto been proved only indirectly, since the organism has not been recovered from the liver.

The patient was a 25-year-old nulligravida who presented because of a 10-day history of abdominal pain in the right upper quadrant. Physical examination revealed tenderness in this area. Bimanual palpation of the internal genitals on the right side was painful. The erythrocyte sedimentation rate was 77 mm per hour.

Laparoscopy disclosed adhesion of the right lobe of the liver to the anterior abdominal wall. During laparoscopy, the adherent area became loose, leaving hemorrhagic spots and fibrinous plaques on the liver surface. The fallopian tubes were red and swollen. Pus appeared in the abdominal orifice of the right tube.

A cannula was inserted through the abdominal wall into the right iliac fossa. A specially designed, sterile-packed, cotton-tipped aluminum probe (30 cm long) (ENT, Medical Wire and Equipment Co., Ltd., Corsham, Wiltshire, England) was introduced through the cannula. After the liver surface was swabbed, the internal genital organs were manipulated. The abdominal orifice of the right fallopian tube was then similarly swabbed.

C. trachomatis was isolated from the cervix and from the liver capsule, but not from the fallopian tube.⁷ *Neisseria gonorrhoeae* was not isolated from any site. On admission the microimmunofluorescent serum IgG antibody titer to *C. trachomatis*¹ had been 1:256; after 13 days it was 1:4096.

We believe that the 30-cm sampling probe can be useful in obtaining specimens at laparoscopy and other types of endoscopy. As compared with other types of widely used swabs, it is comparatively nontoxic to organisms such as *C. trachomatis*.⁷

PÅL WØLNER-HANSEN, M.D.

LARS SVENSSON, M.D.

LARS WESTRÖM, M.D.

University Hospital

S-221 85 Lund, Sweden

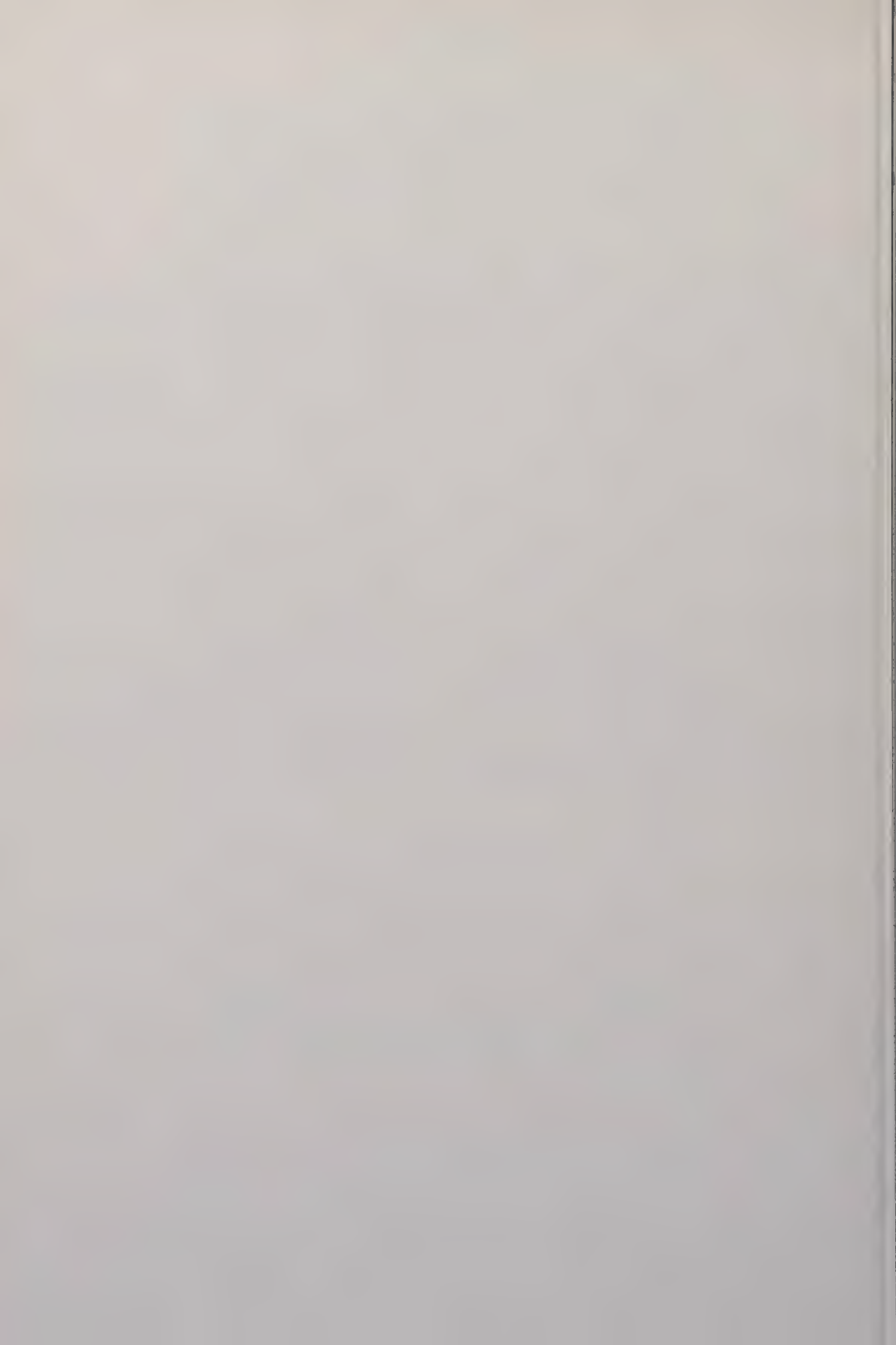
PER-ANDERS MÅRDH, M.D.

Institute of Medical Microbiology

University of Lund

S-223 62 Lund, Sweden

1. Müller-Schoop JW, Wang SP, Münzinger J, Schläpfer HU, Knoblauch M, Amman RW. *Chlamydia trachomatis* as a possible cause of peritonitis and perihepatitis in young women. *Br Med J*. 1978; 1:1022-4.
2. Wølner-Hansen P, Weström L, Mårdh P-A. Perihepatitis and chlamydial salpingitis. *Lancet*. 1980; 1:901-4.
3. Wang SP, Eschenbach D, Holmes KK, Wager G, Grayston JT. *Chlamydia trachomatis* infection in Fitz-Hugh-Curtis syndrome. *Am J Obstet Gynecol*. 1980; 138:1034-8.
4. Paavonen J, Saikku P, Wang SP, von Knorring J, Aho K. Frequent association of *Chlamydia trachomatis* infection with Fitz-Hugh-Curtis syndrome. *J Infect Dis*. (in press).
5. Dalaker K, Gjønnaess H, Kvile G, Urnes A, Ånestad G, Bergan T. *Chlamydia trachomatis* as a cause of acute perihepatitis associated with pelvic inflammatory disease. *Br J Vener Dis*. 1981; 57:41-3.
6. Gjønnaess K, Dalaker K, Ånestad G, Mårdh P-A, Kvile G, Bergan T. Pelvic inflammatory disease: etiological studies with emphasis on chlamydial infection. *Obstet Gynecol*. (in press).
7. Mårdh P-A, Zeeberg B. The toxic effect of sampling swabs and transportation test tubes on the formation of intracytoplasmic inclusions of *Chlamydia trachomatis* in tissue cell cultures. *Br J Vener Dis*. 1981; 57:268-72.



Laparoscopy in Women With Chlamydial Infection and Pelvic Pain: A Comparison of Patients With and Without Salpingitis

PÅL WØLNER-HANSEN, MD, PER-ANDERS MÅRDH, MD,
LARS SVENSSON, MD, AND LARS WESTRÖM, MD

A review was made of clinical and laboratory findings in 104 women who, during 1978 to 1981, were subjected to laparoscopy because of symptoms suggestive of acute salpingitis, and who harbored *Chlamydia trachomatis* but not *Neisseria gonorrhoeae* in the genital tract. The patients with acute salpingitis ($N = 76$) did not differ significantly from those with visually normal fallopian tubes ($N = 28$) in regard to age distribution, parity, contraceptive method used, proportion of women with urethritis symptoms, increased vaginal discharge, vomiting, diarrhea, elevated rectal temperature, elevated white blood cell count, and palpable pelvic masses. The acute salpingitis patients more often had irregular bleeding and an elevated erythrocyte sedimentation rate, whereas the patients without acute salpingitis more often had a short history of pelvic pain. The two groups overlapped considerably with respect to the number of symptoms and clinical signs of pelvic infection. The results emphasize the value of laparoscopy in the diagnosis or exclusion of a tubal infection in association with a chlamydial genital infection and pelvic pain, even if there are comparatively few additional symptoms of ascending infection. (*Obstet Gynecol* 61:299, 1983)

A chlamydial infection of the cervical epithelium can spread canalicularly through the genital tract via the endometrium into the fallopian tubes and cause salpingitis.¹⁻³ Infertility is one sequela to acute salpingitis, but not to cervicitis/endometritis.⁴ Although cervicitis/endometritis/salpingitis are steps of one and the same disease process and are treated alike, it is important that the patient know whether or not the infection carries any significant threat to her future fecundity. It

seems important therefore to evaluate whether it is possible to distinguish cervicitis/endometritis from salpingitis using conventional clinical parameters.

The present retrospective study compares clinical and laboratory parameters in chlamydial genital infections associated with pelvic pain and adnexal tenderness in women with and without laparoscopic signs of acute salpingitis.

Materials and Methods

The relevant details of all women who had been subjected to laparoscopy because of a provisional diagnosis of acute pelvic inflammatory disease at the University Hospital, Lund, Sweden, during 1978 to 1981 were extracted from the records. The minimum criteria for performing laparoscopy were: acute lower abdominal pain, abnormal tenderness over the adnexa at pelvic examination, and purulent vaginal discharge and/or irregular uterine bleeding. The minimum criteria for the visual diagnosis of acute salpingitis were: hyperemia of the tubal surface, edema of the tubal wall, purulent discharge from the abdominal orifice, and/or fresh peritubal adhesions. The study included only women who were culture-positive for *Chlamydia trachomatis* and culture-negative for *Neisseria gonorrhoeae* from the genital tract.

One hundred four women fulfilled these inclusion criteria. From their records the authors extracted information regarding age, parity, contraceptive method, symptomatology (ie, duration of pelvic pain before admission, abnormal vaginal discharge, irregular uterine bleeding that had started in association with the disease, vomiting, diarrhea, and urethritis symptoms), palpatory and laboratory findings (ie, rectal temperature in the first morning after admission, erythrocyte

From the Department of Obstetrics and Gynecology, University Hospital; and the Institute of Medical Microbiology, University of Lund, Lund, Sweden.

Supported by a grant from WHO and by grant no. 16X-4509 from the Swedish Medical Research Council.

sedimentation rate, white blood cell count, and the results of wet smear microscopy of cervical secretion).

All women in the study were treated with a chlamydiostatic drug as soon as the laparoscopy had been performed. Specimens for isolation of *C trachomatis* and *N gonorrhoeae* were collected from all patients by rotating swabs in the cervical canal and the urethra after exposing the cervical os and urethral orifice by inserting a sterile speculum into the vagina. Rectal specimens were also studied for gonococci. In 15 patients cultures were made of contents that had been collected from the uterine cavity by a protected transcervical aspiration method, as described in detail elsewhere.⁵ Specimens were obtained in 21 patients from the abdominal orifice of a fallopian tube for culturing as described previously.⁶ The techniques used for the isolation and identification of *C trachomatis*⁷ and *N gonorrhoeae*⁸ were those described earlier. Description of the laparoscopic technique has been given elsewhere.⁹ A modified microimmunofluorescence test was used for IgM and IgG antibodies to *C trachomatis*.¹⁰ The χ^2 test with Yate correction was used to compare frequencies; the Wilcoxon-Mann-Whitney rank sum test was used to compare means.

Results

Laparoscopy revealed acute salpingitis in 76 (73%) and visually normal fallopian tubes in 28 (27%) of the 104 patients included in the study. Of the 76 acute salpingitis patients, 15 were classified as having severe, 16 as having moderately severe, and 45 as having mild acute salpingitis.⁹ Perihepatitis and periappendicitis (histologically verified) were found in six (7.9%), and eight (10.5%), respectively, of the acute salpingitis patients. Of the 28 patients without acute salpingitis, three had mild endometriosis, one of whom also had a corpus luteum hematoma; one patient had mesenterial lymphadenitis and another had a corpus luteum hematoma alone. None of the patients without acute salpingitis had signs of periappendicitis or perihepatitis. No significant complications occurred during or after any of the laparoscopies.

Table 1. Isolates of *C trachomatis* in 104 Women Who Had Pelvic Pain and Adnexal Tenderness

Site	Salpingitis		Nonsalpingitis	
	No. of isolates	Percent	No. of isolates	Percent
Cervix	73/76	96	28/28	100
Endometrium	7/11	64	1/4	25
Fallopian tubes	4/21	19		
Liver	1/3	33		

Table 2. Comparison of Symptoms and Signs in Women With Acute Salpingitis and Those With Visually Normal Tubes

Characteristics	Salpingitis (N = 76)		Nonsalpingitis (N = 28)		Difference (P)
	No.	Percent	No.	Percent	
Pain < 4 days	18/76	24	15/28	54	<.01
Pain 7-14 days	25/76	33	3/28	11	<.05
Irregular bleeding	38/76	50	5/28	18	<.01
Increased discharge	52/76	68	16/28	57	NS
Urethritis symptoms	14/68	21	9/25	36	NS
Nausea, vomiting	11/35	31	9/15	60	NS
Diarrhea	2/44	5	2/15	13	NS
Palpable mass	18/76	24	6/28	21	NS
Temperature $\geq$ 38C	18/76	24	6/28	21	NS
Purulent discharge	48/55	87	18/24	75	NS
ESR > 15 mm/hr	58/76	76	9/28	31	<.0005
WBC > 10 ¹⁰ /liter	21/48	44	6/19	32	NS

ESR = erythrocyte sedimentation rate; WBC = white blood cell count; NS = not significant.

C trachomatis was recovered from the cervix in 101 of the 104 patients, from the endometrium in eight of 15, from the fallopian tubes in four of 21, and from the liver surface in one of three from whom culture specimens were obtained (Table 1). In ten patients the microorganism was isolated from more than one of these anatomic sites. Three patients harbored chlamydiae only in the fallopian tubes, the endometrium, or the endometrium plus the fallopian tubes, respectively.

Twenty-eight of the 45 acute salpingitis patients and one of the nine patients without acute salpingitis from whom paired sera were obtained had a four-fold or greater change in titer of IgG antibodies to *C trachomatis*. Six of the other 31 acute salpingitis patients, but none of the other ten patients without acute salpingitis from whom at least one serum sample had been obtained, had one titer of such antibodies greater than or equaling 1:512. Serum samples had not been obtained from one patient who harbored chlamydiae in the tubes. Thus a chlamydial cause of the salpingitis was proved in 35 of the 76 acute salpingitis patients.

The ages of the 104 women varied from 15 to 36 years (mean, 21.6 years). The mean age of the acute salpingitis patients (22.2 years) and those without acute salpingitis (20.1 years) did not differ significantly. Of the women in either group, 40% had at some time been pregnant. More acute salpingitis patients were intrauterine contraceptive device users than patients without acute salpingitis (36 and 14%, respectively), whereas more patients without acute salpingitis used oral contraceptives than did acute salpingitis patients (54 and 38%, respectively). The differences

were not significant. The two groups did not differ significantly regarding frequency of increased vaginal discharge, purulent discharge, urethritis symptoms, vomiting, diarrhea, elevated rectal temperature (38C or above), elevated white blood cell count (above 10¹⁰/liter), or palpable pelvic masses (Table 2).

Significantly more patients without than with acute salpingitis reported a short history of pelvic pain, ie, less than four days before admission (54 and 24%, respectively, $\chi_1^2 = 9.87, P < .01$). Acute salpingitis patients had had pelvic pain for seven to 14 days before seeking medical advice in the authors' clinic significantly more often than patients with apparently normal fallopian tubes (33 and 11%, respectively, $\chi_1^2 = 4.05, P < .05$).

Irregular uterine bleeding associated with the disease was reported by half the acute salpingitis patients. Approximately one of six patients without acute salpingitis had similar occurrences ($\chi_1^2 = 7.44, P < .01$). The incidence of elevated erythrocyte sedimentation rate (15 mm/hr or more) was significantly higher in the acute salpingitis patients than in the patients without acute salpingitis ($\chi_1^2 = 15.55, P < .0005$).

When comparing the 45 acute salpingitis patients who had only mild inflammatory changes of the fallopian tubes with the patients without acute salpingitis, the frequencies of a short history of pelvic pain ($\chi_1^2 = 10.4, P < .005$), occurrence of irregular bleeding ($\chi_1^2 = 5.05, P < .05$), and elevated erythrocyte sedimentation rate ($\chi_1^2 = 5.99, P < .05$) were found to differ significantly (Table 3).

When comparing the number of symptoms and signs of pelvic inflammatory disease that were found in addition to pelvic pain and adnexal tenderness, a

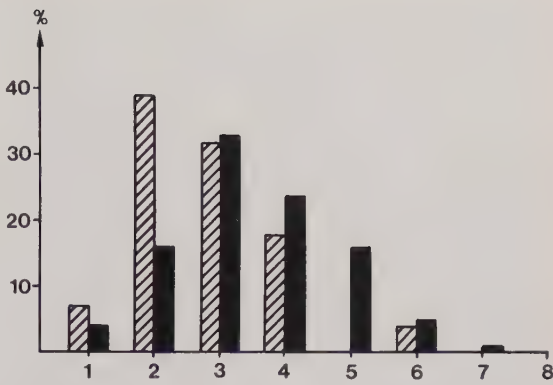


Figure 1. Distribution of patients with acute salpingitis and patients with visually normal fallopian tubes, all of whom harbored *Chlamydia trachomatis* in the genital tract, with the number of clinical signs and symptoms of pelvic inflammatory disease (N = 8; see text) that were found in conjunction with pelvic pain and adnexal tenderness. Solid bars = acute salpingitis; hatched bars = nonsalpingitis.

great deal of overlap between those with and without acute salpingitis was demonstrated (Figure 1). In both groups the median patient had three of the eight symptoms considered (irregular uterine bleeding, increased vaginal discharge as reported by the patient, purulent discharge observed on microscopy of wet smear, urethritis symptoms, elevated erythrocyte sedimentation rate, elevated white blood cell count, fever, and palpated pelvic masses).

The acute salpingitis:non-acute salpingitis ratio in relation to the number of symptoms is shown in Figure 2. It appears that half the patients with only one or two symptoms in addition to pelvic pain and adnexal tenderness had laparoscopically verified acute salpingitis. Moreover, at least five symptoms seem to be needed for diagnosing acute salpingitis clinically with a fair amount of certainty.

Table 4 shows the frequency of some combinations of symptoms. The classically described combination of clinical symptoms and signs, ie, purulent discharge, fever, and elevated erythrocyte sedimentation rate in addition to pelvic pain and adnexal tenderness, was found in only seven (9%) of the 76 patients with acute salpingitis and in two (7%) of the patients with visually normal tubes.

Discussion

It has been suggested that a laparoscopic diagnosis of the absence of acute salpingitis might include some cases with an endosalpingitis.¹¹ As pointed out earlier, however, the most important prerequisite for a laparoscopic diagnosis of salpingitis is the presence of a purulent discharge visible on the mucosal surface of

Table 3. Comparison of Symptoms and Signs in Women With Mild Salpingitis and Those With Visually Normal Tubes

Characteristics	Salpingitis (N = 76)		Nonsalpingitis (N = 28)		Differ- ence (P)
	No.	Percent	No.	Percent	
Pain < 4 days	9/45	20	15/28	54	<.005
Pain 7-14 days	15/45	33	3/28	11	NS
Irregular bleeding	21/45	47	5/28	18	<.05
Increased discharge	33/45	73	16/28	57	NS
Urethritis symptoms	8/35	23	9/25	36	NS
Nausea, vomiting	8/22	36	9/15	60	NS
Diarrhea	2/26	8	2/15	13	NS
Palpable mass	8/45	18	6/28	21	NS
Temperature ≥ 38C	9/45	20	6/28	21	NS
Purulent discharge	32/36	89	18/24	75	NS
ESR > 15mm/hr	29/45	64	9/28	31	<.05
WBC > 10 ¹⁰ /liter	9/30	30	6/19	32	NS

ESR = erythrocyte sedimentation rate; WBC = white blood cell count; NS = significant.

Table 4. Various Combinations of Symptoms and Signs That Were Found in Conjunction With Pelvic Pain and Adnexal Tenderness

Characteristic	Salpingitis (N = 76)		Nonsalpingitis (N = 28)		P
	No.	Percent	No.	Percent	
Purulent discharge, fever, ESR > 15 mm/hr	7	9	2	7	NS
Fever, ESR > 15 mm/hr	14	18	3	10	NS
Irregular bleeding, fever, ESR > mm/hr	8	11	0	0	NS
Irregular bleeding, ESR > 15 mm/hr	33	43	2	7	<.005

ESR = erythrocyte sedimentation rate; NS = not significant.

the fimbriated end of the tube.⁹ Such a discharge is present also in endosalpingitis.¹² Furthermore, there is a strong correlation between presence or absence of specific genital isoenzymes in peritoneal fluid and the laparoscopic diagnosis of absence or presence of a tubal infection.¹³

The present retrospective study shows that patients with pelvic pain and adnexal tenderness who harbor *C trachomatis* in the genital tract may or may not have salpingitis as indicated by laparoscopy. Thus, of the 104 women studied, 25% (28 cases) had visually normal fallopian tubes. Regarding the number of symptoms and signs apart from pelvic pain and adnexal tenderness, 20% of the acute salpingitis patients had only one or two symptoms, whereas more than half the patients without acute salpingitis had three or more symptoms. Thus the study confirms the comparatively mild clinical course of chlamydia-associated salpingitis as compared to gonococcal and anaerobic salpingitis.¹⁴ The study also demonstrates that in a single case it is difficult to determine whether or not the tubes are involved in the infectious process by use of clinical criteria only. A more restricted use of laparoscopy would reduce the number of patients without acute salpingitis subjected to the procedure, but a proportion of patients with acute salpingitis would remain undiagnosed.

As to the differences between salpingitis and nonsalpingitis patients, the authors found a short period of abdominal pain before consultation to be negatively correlated, and an elevated erythrocyte sedimentation rate to be positively correlated to acute salpingitis. This indicates that the time is an important variable in the establishment of a tubal infection. If so, the observation underscores the importance of early diagnosis and treatment. The progress of the infectious process in the

patients without acute salpingitis could not be evaluated because all those patients were treated with chlamydia-static antibiotics.

In the present study of chlamydial genital infections, irregular uterine bleeding was reported significantly more often by the patients with acute salpingitis than by the nonsalpingitis patients. This is contrary to earlier reports from the authors' hospital showing that in young women with genital infections, 33 to 50% reported to have had this symptom regardless of whether the fallopian tubes were involved in the infectious process.⁹ In those studies chlamydial cultures had not been performed. It has been suggested that metrorrhagic bleeding might be a symptom of endometritis.¹⁵ However, attempts to correlate such bleeding with culture and/or histopathologic diagnosis of endometritis have failed.⁵ This might be caused by the often patchy character of the endometrial inflammatory reactions.¹⁶ Evidently, more research is needed to establish the frequency and role of *C trachomatis* in endometritis.

One of the selective criteria in the present study was pelvic pain with adnexal tenderness. The study confirms that the fallopian tubes need not be involved in the inflammatory process of genital infection for such symptoms to occur. The cause of the pain and tenderness is unclear in the 24 patients without acute salpingitis who had no pathologic intrapelvic findings at laparoscopy. One could hypothesize that *C trachomatis* might spread not only canalicularly from the cervix, via

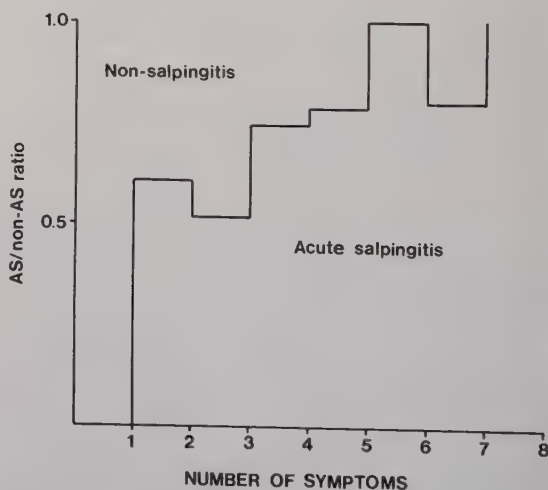


Figure 2. Patients with acute salpingitis in comparison to patients with visually normal fallopian tubes, all of whom harbored *Chlamydia trachomatis* in the genital tract, with the number of clinical signs and symptoms of pelvic inflammatory disease (N = 8) in addition to pelvic pain and adnexal tenderness.

endometrium, but also by the parametrial lymphatics that pass along the uterine vessels, thus causing parametrial lymphangitis. Grivet monkeys, experimentally infected in the uterine cavity with *Mycoplasma hominis*, develop a parametritis that might be a manifestation of such lymphatic spread,¹⁷ but parametritis has not been observed in grivet monkeys infected with *C trachomatis*. Whether or not parametritis might be a manifestation of genital chlamydial infection in humans remains to be clarified. If so, parametric lymphangitis might explain the tenderness over the adnexal region as well as the pain reported on dislocation of the uterus in women with and without acute salpingitis.

If it is important to assess the impact of a genital chlamydial infection on the future fecundity of the patient, the results of the present study underscore the need for laparoscopy in women with such infections plus pelvic pain and adnexal tenderness, even if few additional symptoms and signs of pelvic inflammatory disease are present.

References

1. Hamark B, Brorsson J-E, Jeansson S, et al: Bacteriological cultures from the endometrial cavity in patients with acute salpingitis, Abstracts of the XXI Scandinavian Congress of Obstetrics and Gynecology, Gothenburg, Sweden. Acta Obstet Gynecol Scand (Suppl) 93:55, 1980

2. Mårdh P-A, Møller BR, Ingerslev HJ, et al: Endometritis caused by *Chlamydia trachomatis*. Br J Vener Dis 58:191, 1981

3. Gump DW, Dickstein S, Gibson M: Endometritis related to *Chlamydia trachomatis* infection. Ann Intern Med 95:61, 1981

4. Weström L: Effect of acute pelvic inflammatory disease on fertility. Am J Obstet Gynecol 121:707, 1975

5. Wølner-Hanssen P, Mårdh P-A, Møller BR, et al: Endometrial infection in women with chlamydial salpingitis. Sex Transm Dis 9:84, 1982

6. Mårdh P-A, Ripa KT, Svensson L, et al: *Chlamydia trachomatis* infection in patients with acute salpingitis. N Engl J Med 196:1377, 1977

7. Ripa KT, Mårdh P-A: Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. J Clin Microbiol 6:328, 1977

8. Mårdh P-A, Mårtensson D, Soltesz LV: An effective, simplified medium for the culture of *Neisseria gonorrhoeae*. Sex Transm Dis 5:10, 1978

9. Jacobsson L, Weström L: Objectivized diagnosis of acute pelvic inflammatory disease. Am J Obstet Gynecol 105:1088, 1969

10. Trehan JD, Darougar S, Jones BR: Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. J Clin Pathol 30:510, 1977

11. Eschenbach DA, Holmes KK: Acute pelvic inflammatory disease: Current concepts of pathogenesis, etiology, and management. Clin Obstet Gynecol 18:33, 1975

12. Weström L, Mårdh P-A: Acute salpingitis, Sexually Transmitted Diseases. Section V, Chapter 57. Edited by KK Holmes, P-A Mårdh, PF Sparling, et al. New York, McGraw-Hill, 1983

13. Weström L, Skude G, Mårdh P-A: Amylases of the genital tract. II. Peritoneal fluid isomylases in acute salpingitis. Am J Obstet Gynecol 126:657, 1976

14. Svensson L, Weström L, Ripa KT, et al: Differences in some clinical and laboratory parameters in acute salpingitis related to culture and serologic findings. Am J Obstet Gynecol 138:1017, 1980

15. Greenwood SM, Moran JJ: Chronic endometritis: Morphologic and clinical observations. Obstet Gynecol 58:176, 1981

16. Runge H: Biologie und Pathologie des Weibes, Endometritis. Vol 5. Edited by L Seitz, AT Amreich. Wien, Urban & Schwarzenberg, 1953, pp 445-450

17. Møller BR, Freundt EA, Mårdh P-A: Experimental pelvic inflammatory disease provoked by *Chlamydia trachomatis* and *Mycoplasma hominis* in grivet monkeys. Am J Obstet Gynecol 138:990, 1980

Address reprint requests to:
Pål Wølner-Hanssen, MD
Department of Obstetrics and Gynecology
University Hospital
S-221 85 Lund, Sweden

Submitted for publication April 21, 1982.
Revised September 17, 1982.
Accepted for publication September 20, 1982.

Copyright © 1983 by The American College of Obstetricians and Gynecologists.



Second-Look Laparoscopy After Acute Salpingitis

PÅL WØLNER-HANSEN, MD, AND LARS WESTRÖM, MD

Patients with a laparoscopically verified first episode of acute salpingitis were offered a second-look laparoscopy to determine the effectiveness of the treatment regimen in terms of anatomic end result as well as to correlate the anatomic end result with future fertility. Until now, 13 women have been subjected to such relaparoscopy 16 to 33 weeks after the acute illness. At the acute stage of the disease, 11 of the women had serologic and/or cultural evidence of genital infection with *Chlamydia trachomatis*, three of whom also had *Neisseria gonorrhoeae*. All were culture-negative after treatment. In eight patients relaparoscopy showed that previously adherent adnexa were apparently normal, whereas in two patients a deterioration of the laparoscopic findings was found. Two patients had bilateral occluded tubes, for a preliminary infertility rate of 15%. These preliminary observations suggest that second-look laparoscopy after acute salpingitis might be a useful method for early objective evaluation of treatment results. (*Obstet Gynecol* 61:702, 1983)

Acute salpingitis is generally a benign infection; complete clinical recovery usually results even without antibiotic treatment.¹ However, even after apparently appropriate treatment, a proportion of the women are rendered infertile because of postinflammatory tubal damage.² Evaluations of the effect of various treatment regimens for salpingitis have usually been limited to the acute episode. The final outcome regarding impairment of tubal function after the infection does not become apparent until the woman has exposed herself to a chance of achieving a pregnancy, ie, often not until years after the infection.³ As all treatment recommendations for acute salpingitis must also take into consideration the long-term effects, there is an obvious need for objective methods of early evaluation of the postinflammatory state of the tubes.

Here the authors present a preliminary report from an ongoing study in which they use second-look laparoscopy for a first and comparatively early evalua-

tion of the treatment results. The purposes of the study are 1) to elucidate the early anatomic results of treatment and 2) to follow the women prospectively to correlate the anatomic end result of tubal function with pregnancy potential.

Materials and Methods

Patients with a laparoscopically verified first episode of acute salpingitis are offered a second-look laparoscopy to be performed approximately six months after the acute disease.

On admission for the salpingitis, specimens for isolation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were collected by rotating swabs in the cervical canal and the urethra. Rectal specimens were also studied for gonococci. Using a protected transcervical aspiration method,⁴ culture specimens were obtained from the uterine cavity.

During laparoscopy, culture specimens were collected from the abdominal orifice of the fallopian tubes as described previously,⁵ or, when the abdominal orifices were inaccessible because of adhesions, by transabdominal puncture of one of the tubes and aspiration of luminal contents under laparoscopic control. A description of the laparoscopic technique used and staging of the inflammatory reactions of the tubes has been given elsewhere.²

After the initial laparoscopy, all women were hospitalized for approximately ten days and treated with antibiotics (Table 1).

Serum samples for determination of the titers of IgG antibodies to *C trachomatis* were obtained on admission and ten to 14 days later.

Contact-tracing and treatment of sexual contacts were performed if the patient harbored sexually transmitted microorganisms in the genital tract.

On the admission day for second-look laparoscopy, culture specimens were collected from cervix, urethra, and rectum as described above, and serum samples were obtained for determination of the titers of IgG

From the Department of Obstetrics and Gynecology, University Hospital, Lund, Sweden.

Table 1. Treatment Regimen Instituted at the Time of Acute Salpingitis as Well as the Findings at Acute-Stage and Second-Look Laparoscopy

Patient no.	Treatment	Acute stage		Second-look	
		Tubal occlusion left/right	Adnexal adhesions left/right	Tubal occlusion left/right	Adnexal adhesions left/right
1	Tetracycline	0/0	0/0	0/0	0/0
2	Rosaramicin	0/0	0/0	0/0	0/0
3	Tetracycline	0/0	0/+	0/0	0/0
4	Doxycycline	?/0	+/0	+/+	+/+
5	Doxycycline	0/0	+/+	0/0	0/0
6	Doxycycline	0/0	+/+	0/0	+/+
7	Doxycycline	0/0	+/+	0/0	0/0
8	Tetracycline	?/0	+/+	0/0	0/0
9	Rosaramicin	0/?	+/+	0/0	0/0
10	Rosaramicin	?/?	+/+	+/0	0/+
11	Rosaramicin	?/?	+/+	+/+	+/+
12	Tetracycline	0/0	0/0	0/0	+/0
13	Doxycycline	0/0	+/+	0/0	0/0

0 = patent abdominal orifice or no adhesions; + = occluded tube or adnexal adhesions; ? = unknown because of inaccessibility.
Dosages: tetracycline hydrochloride, 2 g/day, rosaramicin, 2 g/day; doxycycline, 200 mg/day.

antibodies to *C trachomatis*. Intraabdominal cultures were not obtained during the second-look laparoscopy.

Results

Until now 13 patients have been subjected to relaparoscopy 16 to 33 weeks after the acute illness. No complications were encountered during the 26 laparoscopies performed in this study.
After the acute stage treatment, cervical control cultures had yielded no growth of *N gonorrhoeae* or *C trachomatis*. None of the patients had experienced any genital infections between the laparoscopies, and

of 11 patients from whom cervical culture specimens were collected on admission for relaparoscopy, none harbored sexually transmitted organisms..
The results of the initial laparoscopy as well as the results of the etiologic studies are presented in Table 2. Eleven of the 13 patients had cultural signs of *C trachomatis* infection; nine of these also had serologic evidence for such infection. Three of the 11 also had *N gonorrhoeae* in the cervix.
In Table 2, the serologic results at the time of relaparoscopy are shown. Eight of the patients had levels of IgG antibodies to *C trachomatis* which were 1:64 or greater during the acute stage of disease. All seven patients who had IgG antibody to *C trachomatis*

Table 2. Inflammatory Changes and Culture Results at the Time of Acute Salpingitis as Well as IgG Antibody Titers to *C trachomatis*

Patient no.	Inflammatory changes	Culture results		Microimmunofluorescent IgG antibody titer to <i>C trachomatis</i>		
		Cervix/endo-metrium	Fallopian tubes	Acute	Convalescence	Relaparoscopy
1	Mild	0	0	<8	<8	<8 (21)
2	Mild	0	0	<8	<8	NE (30)
3	Mild	c	0	512	8192	8192 (28)
4	Moderate	c	0	256	1024	512 (19)
5	Mild	c,g	0	<8	32	NE (26)
6	Mild	c	0	4096	8192	512 (16)
7	Mild	c	0	128	4096	512 (24)
8	Moderate	c	c	512	4096	512 (33)
9	Moderate	c,g	0	128	64	64 (27)
10	Moderate	c,g	c	32	256	NE (33)
11	Moderate	c	NE	1024	256	NE (26)
12	Mild	c	0	16	32	128 (21)
13	Mild	c	NE	1024	4096	256 (31)

c = *C trachomatis*; g = *N gonorrhoeae*; 0 = no organisms recovered; NE = not examined; () = weeks between first and second laparoscopy.

during the acute stage of disease and who were serologically tested at the time of relaparoscopy still had IgG titers of 64 or greater. At this third sampling five of the patients had an IgG antibody titer higher than or equal to the acute stage titer.

Table 1 discloses the results of the first and second laparoscopy in terms of tubal occlusion and adnexal adhesions as well as the treatment regimens instituted. No patient had obviously occluded tubes at the acute-stage laparoscopy, but in five of the 13 patients the fimbriated end of one or both tubes was not accessible because of heavy adhesions. Patients 1 and 2, the only ones with negative cultures and chlamydial serology, also had the mildest inflammatory changes of the fallopian tubes.

In eight patients, repeat laparoscopy showed that previously adherent adnexa were apparently normal. Two of the 13 patients (nos. 4 and 12) showed a deterioration of the laparoscopic findings since the initial examination. Two patients (15%) had bilateral occluded fallopian tubes at the second-look laparoscopy. Four patients (nos. 1, 3, 5, and 6) have had an intrauterine pregnancy since the relaparoscopy.

Comments

In the present study, a second-look laparoscopy was performed approximately six months after laparoscopically verified acute salpingitis to elucidate the anatomic consequences of the disease. The preliminary findings, after reexamination of 13 patients in this way, indicate that acute-stage adnexal adhesions might disappear after subsidence of the disease. This might be caused partly by manipulations of the adnexa during the initial laparoscopy. But, as such manipulations are minimal, the authors believe that spontaneous absorption is of more importance. The deterioration of the laparoscopic findings since the initial examination in two appropriately treated patients as well as the disappearance of adhesions in eight patients do highlight the fact that the acute laparoscopy gives only a "snapshot" view of a dynamic process.

Despite the relatively high antibody titers to *C trachomatis* at the time of second-look laparoscopy, none of the eight patients studied in this way had any signs of ongoing inflammation of the fallopian tubes. This demonstrates the slow decline of the IgG titer after subsidence of the acute disease. Accordingly, a single IgG antibody titer, even when high, might not alone prove a current pelvic inflammatory disease with *C trachomatis*, as was recently alleged.⁶

Hysterosalpingography was not performed in any of the patients because such a procedure gives informa-

tion only on the lumen of the genital tract, not on adnexal adhesions and other anatomic postinflammatory intrapelvic sequelae. Moreover, hysterosalpingography might falsely indicate bilateral proximal tubal blocks. In the experience of one of the authors (LW), the use of hysterosalpingography caused a flare-up of the tubal infection in 13% of the patients.

Relaparoscopy disclosed bilateral distal occluded tubes in two patients. Although their tubal function has not yet been tested, infertility seems probable. Thus, the preliminary infertility rate (15%) among the patients studied until now is in accordance with earlier findings.²

These preliminary observations suggest that second-look laparoscopy after acute salpingitis might be a useful method for early objective evaluation of treatment results and for providing a possible prognosis of future fertility. Long-term follow-up of the fertility rate in patients subjected to second-look laparoscopy after salpingitis will eventually disclose the prognostic value of the procedure.

References

1. Haffner J: Resultatene av den konservative og operative salpingit-behandling. Nord Med 3:2255, 1939
2. Jacobsson L, Weström L: Objectivized diagnosis of acute pelvic inflammatory disease. Am J Obstet Gynecol 105:1088, 1969
3. Weström L: Effect of acute pelvic inflammatory disease on fertility. Am J Obstet Gynecol 121:707, 1975
4. Wølner-Hanssen P, Mårdh P-A, Möller BR, et al: Endometrial infection in women with chlamydial salpingitis. Sex Transm Dis 9:84, 1982
5. Mårdh P-A, Ripa KT, Svensson L, et al: *Chlamydia trachomatis* infection in patients with acute salpingitis. N Engl J Med 196:1377, 1977
6. Henry-Suchet J, Catalan F, Paris X, et al: Antibody titer to *Chlamydia trachomatis* in acute salpingitis and obstructive sterilities, Chlamydial Infections, Proceedings of the 5th International Symposium on Human Chlamydial Infections. Edited by P-A Mårdh, KK Holmes, JD Oriol, et al. Amsterdam, Elsevier Biomedical Press, 1982, p 183

Address reprint requests to:

Pål Wølner-Hanssen, MD
Department of Obstetrics and Gynecology
University Hospital
S-221 85 Lund, Sweden

Submitted for publication July 23, 1982.

Revised November 3, 1982.

Accepted for publication November 8, 1982.

Copyright © 1983 by The American College of Obstetricians and Gynecologists.

LAPAROSCOPIC FINDINGS AND CONTRACEPTIVE USE IN WOMEN WITH SIGNS AND SYMPTOMS SUGGESTIVE OF ACUTE SALPINGITIS

Pål Wølner-Hanssen, Lars Svensson, Per-Anders Mårdh, Lars Weström

From the Department of Obstetrics and Gynecology, University Hospital, and the Institute of Medical Microbiology, University of Lund, Lund, Sweden

Abstract. Laparoscopic findings in women with clinical signs and symptoms of pelvic inflammatory disease were correlated with contraceptive use in a case-control study. Of the 738 women, 544 (73.7%) had laparoscopic signs of acute salpingitis, whereas 194 (26.3%) had visually normal fallopian tubes. Acute salpingitis was seen in 59.8% of the 286 patients using oral contraceptives in 80.6% of the 227 patients using an intrauterine device (IUD), and in 84.4% of the 225 patients using barrier methods or not using contraceptives (reference group). To estimate the relative risk of acute salpingitis, logistic regression analysis adjusting for age and duration of pain before laparoscopy was used. For oral contraceptive users versus the reference group the adjusted relative risk was estimated at 0.24 (95% confidence interval 0.15 to 0.38, $p < 0.0001$), and for IUD users versus the reference group a relative risk was estimated at 0.83 (95% confidence interval 0.49 to 1.38, $p = 0.46$). The relative risk of salpingitis among oral contraceptive users versus the reference group was 0.22 ($p = 0.005$), and 0.06 ($p = 0.001$) for women infected with *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*, respectively. In patients with cervicitis, spread of the inflammation to the fallopian tubes seems to be inhibited in oral contraceptive users.

Pelvic inflammatory disease has been defined as "the acute clinical syndrome attributed to ascending spread of microorganisms (unrelated to pregnancy or surgery) from the vagina and cervix to the endometrium, fallopian tubes and/or contiguous structures" (1). Although cervicitis/endometritis/salpingitis are stages in the ascent of infection, in many cases it is difficult to establish the level of genital tract infection based on clinical criteria alone (2, 3). Previous studies have indicated that the risk of ascending infection is increased in sexually active intrauterine device (IUD) users and diminished in oral contraceptive users, as compared with women using barrier methods or not using contraceptives (4, 5, 6, 7, 8, 9). In most of these studies, the tubes were not visualized to establish the

diagnosis of pelvic inflammatory disease. In one study the pelvic inflammatory disease patients had been subjected to laparoscopy, whereas the control subjects were not (6).

In the present paper the authors studied the contraceptive method used at the time of onset of abdominal pain in women with laparoscopic signs of salpingitis. In contrast to other studies the control group consisted of women with acute lower abdominal pain who were thought to have acute salpingitis, but who had normal fallopian tubes as observed by laparoscopy.

METHODS

The study patients comprised all 15 to 34-year-old women who underwent laparoscopy because of a provisional clinical diagnosis of pelvic inflammatory disease from January 1975 through March 1983 at the University Hospital in Lund. The minimum criteria for performing laparoscopy were acute lower abdominal pain, abnormal tenderness over the adnexa at pelvic examination, and purulent vaginal discharge or irregular uterine bleeding that had started in association with the disease. Excluded from the study were women with more than 3 weeks of abdominal pain, puerperal or postoperative infection (including infection that occurred within four weeks after IUD insertion or legal abortion), a previous history of pelvic inflammatory disease and women in whom laparoscopy revealed other gynecologic or surgical diseases or from whom no reliable information regarding contraceptive use was obtained. The inclusion criteria were fulfilled by 738 women, whereas 293 were excluded for the above-mentioned reasons. Of the excluded women 85 used oral contraceptives, 125 used IUDs, and 83 used barrier methods or no contraceptives.

The technique of laparoscopy is described elsewhere (2). The minimum criteria for the visual diagnosis of acute salpingitis were hyperemia of the tubal surface, plus edema of the tubal wall, and purulent discharge from the abdominal orifice when it was still patent and/or recent, ie, easily breakable, nonfibrotic, peritubal adhesions (2). The operation was performed within 24 hours of admission and before antibiotics had been administered.

Table I. Comparison of laparoscopic findings with contraceptive use in 738 women with signs and symptoms suggestive of acute salpingitis.

Laparoscopic findings	Contraceptive method				Reference*	
	Oral contraceptive	(%)	Intrauterine device	(%)	(%)	(%)
Salpingitis	171	(59.8)	183	(80.6)	190	(84.4)
Nonsalpingitis	115	(40.2)	44	(19.4)	35	(15.6)

Relative risk of oral contraceptive use versus reference = 0.27 ($X^2_1 = 36.9$, $p < 0.001$)

Relative risk of IUD use versus reference = 0.77 (95% confidence interval 0.47–1.26, $X^2_1 = 1.1$, $p = 0.28$)

Relative risk of oral contraceptive use versus IUD use = 0.36 (95% confidence interval 0.24–0.54, $X^2_1 = 25.7$, $p < 0.0001$)

*Reference = barrier methods or no contraceptives

The patients were divided into three categories according to their contraceptive use:

1. reference group, patients using barrier methods or no contraceptives;
2. IUD users, women using an IUD at the time of onset of pelvic pain. The majority used copper-medicated IUDs. Dalkon shield and progesterone-medicated devices were not used; and
3. oral contraceptive users, women using oral contraceptives at the time of onset of pelvic pain. The vast majority of the women used combination oral contraceptives.

Specimens for the culture of *Neisseria gonorrhoeae* were obtained from the urethra, cervix and rectum of the vast majority of patients. After October 1976, specimens for the culture of *Chlamydia trachomatis* generally were obtained from the cervix. In a small proportion of the patients ($n = 42$), these two microorganisms also were cultured from endometrial specimens. The techniques used for the isolation and identification of these two microorganisms were those described (10, 11).

Logistic regression analysis (12) was used to calculate estimates of relative risk. This procedure allowed for calculation of a relative risk estimate adjusted for the possible confounding variables of age and duration of abdominal pain before laparoscopy.

RESULTS

The laparoscopic findings in the 738 patients are shown in Table I. Five hundred forty-four (73.7%)

women had acute salpingitis, whereas 194 (26.3%) had visually normal tubes. Of the 225 women in the reference group, 190 (84.4%) had laparoscopic signs of acute salpingitis, whereas 35 (15.6%) had nonsalpingitis. The corresponding figures for the 227 patients in the IUD group were 183 (80.6%) and 44 (19.4%), respectively, and for the 286 patients in the oral contraceptive group were 171 (59.8%) and 115 (40.2%), respectively. From these data, the unadjusted relative risk of acute salpingitis for oral contraceptive users as compared with the reference group was estimated at 0.27 (95% confidence interval 0.18 to 0.43, $X^2_1 = 36.9$, $p < 0.0001$). For IUD users as compared with the reference group the unadjusted relative risk of acute salpingitis was estimated to be 0.77 (95% confidence interval 0.47 to 1.26, $p = 0.28$).

Chlamydia trachomatis was recovered from the genital tract of 142 of 373 (38.1%) acute salpingitis patients versus 28 of 162 (17.3%) nonsalpingitis patients ($\chi^2 = 19.5$, $p < 0.001$). *Neisseria gonorrhoeae* was isolated from 101 of 518 (19.5%) acute salpingitis patients versus 16 of 191 (8.4%) nonsalpingitis patients ($\chi^2 = 12.6$, $p < 0.001$). Endometrial aspiration was performed in 22 acute salpingitis and 20 nonsalpingitis patients. *Chlamydia trachomatis* was recovered from the endometrial specimens from 11 of the 22 acute salpingitis patients, and 3 of the 20 nonsalping-

Table II. Comparison of culture results from the genital tract with type of contraceptive use in women with signs and symptoms suggestive of acute salpingitis.

Microorganism	Contraceptive method				Reference*		p
	Oral contraceptive	(%)	Intrauterine device	(%)	(%)	(%)	
<i>C. trachomatis</i>	70/213	(32.9)	51/158	(32.3)	50/158	(31.6)	0.97
<i>N. gonorrhoeae</i>	39/277	(14.1)	37/217	(17.1)	42/215	(19.5)	0.27

C. trachomatis = *Chlamydia trachomatis*; *N. gonorrhoeae* = *Neisseria gonorrhoeae*

Relative risk of oral contraceptive use versus reference = 0.27 ($X^2_1 = 36.9$, $p < 0.001$)

Relative risk of IUD use versus reference = 0.77 (95% confidence interval 0.47–1.26, $X^2_1 = 1.1$, $p = 0.28$)

Relative risk of oral contraceptive use versus IUD use = 0.36 (95% confidence interval 0.24–0.54, $X^2_1 = 25.7$, $p < 0.0001$)

*Reference = barrier methods or no contraceptives

Table III. Comparison of laparoscopic findings and contraceptive use in women with signs and symptoms suggestive of acute salpingitis, who were infected with *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*.

Laparoscopic findings	Contraceptive method					
	Oral contraceptive	(%)	Intrauterine device	(%)	Reference	(%)
<i>Women infected with C. trachomatis*</i>						
Salpingitis	50	(71)	46	(90)	46	(92)
Nonsalpingitis	20	(29)	5	(10)	4	(8)
<i>Women infected with N. gonorrhoeae⁺</i>						
Salpingitis	28	(72)	32	(86)	41	(98)
Nonsalpingitis	11	(28)	5	(14)	1	(2)

C. trachomatis = *Chlamydia trachomatis*; *N. gonorrhoeae* = *Neisseria gonorrhoeae*

Reference = barrier methods or no contraception

*Relative risk for oral contraceptive use versus reference = 0.22 ($X^2 = 7.7$, $p = 0.005$)

⁺Relative risk for oral contraceptive use versus reference = 0.06 ($X^2_1 = 10.7$, $p = 0.0001$); and relative risk of IUD use versus reference group = 0.16 ($X^2_1 = 3.5$, $p = 0.06$)

itis patients. Two of each group harbored *N. gonorrhoeae* in the endometrium. Table II demonstrates that patients in the 3 contraceptive groups were infected equally as often with *C. trachomatis* or *N. gonorrhoeae*. Table III shows the relationship between the laparoscopic findings and contraceptive use in women infected with *C. trachomatis* and in those infected with *N. gonorrhoeae*. The oral contraceptive users had acute salpingitis significantly less often than

did women in the reference group, whether or not they were infected with *C. trachomatis* (relative risk = 0.22, $\chi^2 = 7.7$, $p = 0.005$), or *N. gonorrhoeae* (relative risk = 0.06, $\chi^2 = 10.7$, $p = 0.001$).

Oral contraceptive users were younger than the patients in the reference group ($X^2_1 = 22.4$, $p < 0.0001$), and the latter patients were younger than the IUD users ($X^2_1 = 19.8$, $p < 0.001$) as shown in Table IV. Oral contraceptive users had a shorter history of ab-

Table IV. Age distribution of patients with laparoscopic evidence of acute salpingitis and of those with visually normal fallopian tubes within each contraceptive group.

Age group (y)	Oral contraceptive		Intrauterine device		Reference	
	Acute salpingitis	Non-salpingitis (%)	Acute salpingitis	Non-salpingitis (%)	Acute salpingitis (%)	Non-salpingitis (%)
15-19	93	(54)	46	(40)	38	(21)
20-24	54	(31)	50	(43)	65	(36)
25-29	18	(11)	15	(13)	40	(22)
30-34	7	(4)	4	(4)	40	(22)
Total	172	115	183	44	190	35

Reference = barrier methods or no contraception

Table V. Duration of pelvic pain prior to laparoscopy for patients with laparoscopic evidence of salpingitis and for those with visually normal fallopian tubes within each of the contraceptive groups.

Duration of pelvic pain (d)	Oral contraceptive		Intrauterine device		Reference	
	Acute salpingitis	Non-salpingitis (%)	Acute salpingitis	Non-salpingitis (%)	Acute salpingitis (%)	Non-salpingitis (%)
1-3	49	(29)	48	(42)	56	(31)
4-6	40	(23)	28	(25)	33	(18)
7-9	39	(23)	15	(13)	33	(18)
≥10	43	(25)	23	(20)	61	(33)
1-21	171	114	183	43	190	33

Reference = barrier methods or no contraception

dominal pain as compared with the patients in the reference group ($X^2_3=7.8$, $p<0.05$). The IUD users did not differ from the reference group in this respect ($X^2_3=0.66$) as shown in Table V. Using logistic regression analysis, the authors adjusted for the possible confounding factors of age and duration of pain and arrived at an estimated relative risk of acute salpingitis for oral contraceptive users versus reference group of 0.24 (95% confidence interval 0.15 to 0.38, $p<0.0001$), and for IUD users versus the reference group of 0.83 (95% confidence interval 0.49 to 1.38, $p=0.46$).

The salpingitis/nonsalpingitis ratios among excluded oral contraceptive users and patients in the reference group were similar, i.e. 16:69 and 17:66, respectively. Among excluded IUD users, the salpingitis/nonsalpingitis ratio clearly was higher, i.e. 79:46. No major pre- or postoperative complications attributed to the laparoscopy were encountered in these 738 women.

DISCUSSION

In sexually active women, the risk of contracting pelvic inflammatory disease seems to be influenced by the contraceptive method used. Thus, several studies have indicated an increased risk of pelvic inflammatory disease among IUD users and a decreased risk among oral contraceptive users as compared with women using no contraceptives (4, 5, 6, 7, 8, 9). However, in those studies the fallopian tubes had not been visualized to verify the diagnosis of pelvic inflammatory disease in study patients, (4, 5, 7, 8, 9) and/or to verify normal tubes in subjects (4, 5, 6, 7, 8, 9). The difficulty in clinically diagnosing acute salpingitis with certainty has been demonstrated, (2, 3, 13) and also was indicated in this study. Among the 738 women presenting with signs and symptoms suggestive of pelvic inflammatory disease, 26.3% had visually normal tubes.

To study the impact of contraceptive use on the development of the inflammatory disease, the patients were put into three different groups. Patients using no contraceptives or barrier methods were considered the reference group. The term "reference" was used because the authors believe the further development of a lower genital tract infection in these patients might reflect the natural course of the disease, contrary to what might happen in patients using oral contraceptives or IUDs.

When the laparoscopic findings were correlated with contraceptive use, the fallopian tubes were

found to be visibly involved in the inflammatory process four times as often among women using barrier methods or no contraceptives as compared with women using oral contraceptives. Laparoscopic findings were not significantly different between women using IUDs and women in the reference group. However, it must be remembered that the present patients were selected due to presentation of signs and symptoms suggestive of acute pelvic inflammatory disease. Thus, lack of laparoscopic difference between the IUD group and the reference group does not imply that there is no difference between these two groups with regard to the risk of acquiring pelvic inflammatory disease. The above-mentioned relationships held true for patients who were infected with *C. trachomatis*. Among women infected with *N. gonorrhoeae*, those in the reference group had salpingitis more than 16 times as often as those in the oral contraceptive group. Among women excluded from this study, IUD users clearly were overrepresented in the salpingitis group. A common reason for excluding IUD users was recent insertion of the device with or without simultaneous abortion. If these patients had been included in the data analysis, a difference between IUD users and the reference group with regard to the occurrence of salpingitis might have emerged. However, these patients were excluded because the present study concerned specifically IUD carriage, and not the possible effects of IUD insertion and/or surgical procedures.

Because of their awareness of the increased risk of pelvic inflammatory disease in IUD users from previous epidemiologic studies, physicians in the study area largely have refused younger women this kind of contraception. Thus, the women in the oral contraceptive group were younger than those in the reference group, and the IUD users were older than the reference patients. To eliminate age as a confounding factor, age-adjusted relative risk for acute salpingitis was estimated. However, the reduced risk of the fallopian tubes visibly being involved in the disease in oral contraceptive users still was apparent after this adjustment.

Studies have shown that salpingitis patients usually have a longer history of pain before seeking medical advice than those with visually normal tubes at the time laparoscopy is performed (2, 3). Women using different contraceptive methods might differ with regard to their tolerance of pelvic pain and their alacrity to seek medical advice. Thus, an IUD user might attribute abdominal pain to the device, and an oral con-

traceptive user, familiar with the authors' clinics from yearly renewals of her oral contraceptive prescription, might be more willing to seek advice earlier than a patient from the reference group. However, the apparent protective effect of oral contraceptives against salpingitis seems to be verified by these data even after adjustment for duration of pelvic pain.

It is possible that some of the nonsalpingitis patients in the present study had an inflammation of the mucous membranes in their fallopian tubes at the time laparoscopy was performed. Such a low-grade endosalpingitis might not always produce enough pus to be macroscopically recognized in the abdominal orifice of the tubes. Moreover, neither biopsy nor culture specimens were obtained routinely from the endometrium of the nonsalpingitis patients. Thus, an unknown number of the nonsalpingitis patients might have had endosalpingitis and/or endometritis as factors contributing to their symptomatology.

The major finding in this study that among women with acute lower abdominal pain, adnexal tenderness, and a vaginal discharge or irregular uterine bleeding, oral contraceptive users were less likely to have laparoscopically verified salpingitis than women using other contraceptive methods, has several implications. In other studies there may have been an overrepresentation of oral contraceptive users among women who were clinically diagnosed as having salpingitis without a laparoscopic confirmation. In the present study, twice as many oral contraceptive users (40.2%) as IUD users (19.4%) and almost three times as many oral contraceptive users as users of barrier methods or no contraception (15.6%) did not have the presumptive diagnosis of salpingitis verified when laparoscopy was performed. Thus, the significantly decreased prevalence of oral contraceptive use among women with salpingitis already reported (4, 5, 7, 9) actually may have been decreased further. The second implication is that there is a similar accuracy of the clinical diagnosis among women using IUDs as among women using barrier methods or no contraception. The accuracy of the clinical diagnosis of pelvic inflammatory disease among IUD users has been questioned (9) because of the possibility of overdiagnosis among a group of women with an increased rate of bleeding and pain due to the presence of the device *per se*, and possible increased awareness of both the patient and the physician of the higher risk of pelvic inflammatory disease among IUD users. The third implication is that among women with signs of lower genital tract infection (vaginal discharge

and/or uterine bleeding) as well as abdominal pain and adnexal tenderness, oral contraceptives somehow protect the patient from significant tubal damage. It has been noted (14) that among women with salpingitis, oral contraceptive users have a milder degree of tubal abnormalities, but data from this study imply that oral contraceptives either totally protect against tubal damage in some patients or significantly reduce the tubal damage so that it is not laparoscopically recognized.

The mechanism of the protective effect of oral contraceptives is obscure. The contraceptive effect of the drugs is partly a result of an increased density of the cervical mucus preventing penetration of spermatozoa (15). In vitro studies have shown that microorganisms, e.g., chlamydiae, might attach to spermatozoa and be transported through columns of albumen from hens' eggs (16). In another study it was demonstrated that spermatozoa can facilitate transport of various microorganisms through columns of cervical mucus (17). It has been hypothesized that microorganisms might "hitchhike" with spermatozoa in the genital tract (18). Thus, the high density of cervical mucus caused by the progestagenic component of oral contraceptives might be one reason why their use decreases the risk of acquiring acute salpingitis or is associated with milder disease; perhaps only a reduced number of bacteria can penetrate or be carried through the dense cervical mucous in oral contraceptive users.

There is evidence that cytotoxic cells capable of lysing infected cells may occur in mice infected with *Chlamydia psittaci* (19). Young and Taylor (20) suggested that cell mediated immune response might contribute to the observed damage seen in chlamydial conjunctivitis. However, the response of lymphocytes from oral contraceptive users to phytohemagglutinin seems to be reduced as compared with matched controls who had never used oral contraceptives (21). Thus, another reason for the reduced inflammatory alteration of the fallopian tubes in oral contraceptive users may be a possible inhibition of the cell-mediated immunity in these patients.

Further mechanisms that have been suggested to explain this effect of oral contraceptives are changes in myometrial activity causing decreased propulsion of bacteria up into the tubes, and decreased menstrual bleeding from an atrophic endometrium with subsequent reduced availability of nutrients for bacteria (6).

The evaluation of the endometrium by endometrial

biopsies in patients with mucopurulent cervicitis will be important in future studies to determine whether or not oral contraceptives reduce the rate of salpingitis by reducing the rate of endometritis.

REFERENCES

1. Gregg MB. Morbidity and Mortality Weekly Report, Atlanta, Georgia, U.S. Department of Health and Human Services, Centers for Disease Control, 1982;31:43 Suppl.
2. Jacobson L, Weström L. Objectivized diagnosis of pelvic inflammatory disease. *Am J Obstet Gynecol* 1969;105:1088.
3. Wølner-Hanssen P, Mårdh P-A, Svensson L, Weström L. Laparoscopy in women with chlamydial infection and pelvic pain: A comparison of patients with and without salpingitis. *Obstet Gynecol* 1983;61:299.
4. Wright NH, Laemmle P. Acute pelvic inflammatory disease in an indigent population: An estimate of its incidence and relationship to methods of contraception. *Am J Obstet Gynecol* 1968;101:979.
5. Faulkner WL, Ory HW. Intrauterine devices and acute pelvic inflammatory disease. *JAMA* 1976;235:1851.
6. Weström L, Bengtsson L-P, Mårdh P-A. The risk of pelvic inflammatory disease in women using intrauterine contraceptive devices as compared to non-users. *Lancet* 1976;ii:221.
7. Eschenbach DA, Harnisch JP, Holmes KK. Pathogenesis of acute pelvic inflammatory disease: Role of contraception and other risk factors. *Am J Obstet Gynecol* 1977;128:838.
8. Senanayake P, Kramer DG. Contraception and the etiology of pelvic inflammatory disease: New perspectives. *Am J Obstet Gynecol* 1980;138:852.
9. Edelman DA, Berger GS, Keith L. Current problems in obstetric and gynecology. Vol 5. The Use of IUDs and Their Relationship to Pelvic Inflammatory Disease: A Review of Epidemiologic and Clinical Studies. Edited by Leventhal JM, Hoffman JJ, Keith L, Taylor PJ. Chicago, London: Medical Publishers, Inc., 1982; 5-62.
10. Mårdh P-A, Mårtensson D, Soltesz LV. An effective, simplified medium for the culture of *Neisseria gonorrhoeae*. *Sex Transm Dis* 1978;5:10.
11. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977;6:328.
12. Cox DR. Analysis of binary data. London, Methuen, 1970.
13. Allen LA, Schoon MG. Laparoscopic diagnosis of acute pelvic inflammatory disease. *Br J Obstet Gynecol* 1983;90:966.
14. Svensson L, Weström L, Mårdh P-A. Contraceptives and acute salpingitis. *JAMA* 1984;251:2553.
15. Moghissi KS, Syner FN, McBride LC. Contraceptive mechanism of action of microdose norethindrone. *Obstet Gynecol* 1973;41:585.
16. Wølner-Hanssen P, Mårdh P-A. In vitro tests of the adherence of *Chlamydia trachomatis* to human spermatozoa. *Fertil Steril* 1984;42:102.
17. Toth A, O'Leary WM, Ledger W. Evidence for microbial transfer by spermatozoa. *Obstet Gynecol* 1982; 59:556.
18. Howard TL. Bacterial hitch-hikers. *J Urol* 1971; 106:94.
19. Laemmert JK. Cytotoxic cells induced after *Chlamydia psittaci* infection in mice. *Infect Immun* 1982;35:101.
20. Young E, Taylor HR. Immune mechanisms in chlamydial eye infection: Cellular immune responses in chronic and acute disease. *J Infect Dis* 1984;150:745.
21. Barnes EW, MacCuish AC, Loudon NB, Jordan J, Irvine WJ. Phytohaemagglutinin-induced lymphocyte transformation and circulating autoantibodies in women taking oral contraceptives. *Lancet* 1974;i:898.

Reprint requests:

Dr. Pål Wølner-Hansen
Department of Obstetrics and Gynecology
RH-20, University of Washington
Seattle, Washington 98195
USA

In Vitro Tests of the Adherence of *Chlamydia trachomatis* to Human Spermatozoa

PÅL WOLNER-HANSSEN, M.D., AND PER-ANDERS MÅRDH, M.D.



Reprinted from July 1984 Fertility and Sterility

PUBLISHED MONTHLY BY THE AMERICAN FERTILITY SOCIETY, BIRMINGHAM, ALABAMA

Copyright© 1983 The American Fertility Society

In vitro tests of the adherence of *Chlamydia trachomatis* to human spermatozoa

Pål Wølner-Hanssen, M.D.*†

Per-Anders Mårdh, M.D.‡

University Hospital and University of Lund, Lund, Sweden

Chlamydia trachomatis is one of the most common causative agents of ascending genital infection. The mechanisms by which microorganisms spread to the upper genital tract are, however, by and large still unknown. Attachment of serovars D, H, and I of *C. trachomatis* to human spermatozoa was observed in in vitro experiments. The specimens were studied by immunofluorescence tests using monoclonal antibodies to *C. trachomatis* and transmission electron microscopy. The adherence of chlamydiae to spermatozoa was enhanced by increasing the acidity of the test environment, that is, from pH 8.0 to 4.0, by increasing the concentration of chlamydial cells in relation to spermatozoa, and by increasing the incubation time (up to 1 hour). Sperm penetration tests, using capillary tubes filled with albumen from hen's eggs, revealed that spermatozoa, when progressing forward, can carry chlamydiae attached to them. *Fertil Steril* 42:102, 1984

Chlamydia trachomatis is a common cause of genital infection, both in the female¹ and in the male.² It can spread canalicularly from the cervix to the uterine cavity and the fallopian tubes, causing endometritis^{3,4} and salpingitis.⁵ Chlamydial infection in the male urethra can be complicated by epididymitis.⁶ Experimental studies in animals suggest that the organism can spread via the spermatic cord to the epididymis.⁷ Mechanisms involved in the ascension of chlamydiae are obscure.

Microorganisms such as *Neisseria gonorrhoeae*,⁸⁻¹⁰ *Escherichia coli*,⁸ and *Ureaplasma*

*urealyticum*¹¹ have the ability to adhere to spermatozoa. The possibility that microorganisms, e.g., gonococci, attached to spermatozoa might be transported from the seminal vesicles to the epididymis, thus giving rise to epididymitis, has been considered.¹² The purpose of the present study was to establish, using an in vitro model, whether chlamydiae can also adhere to spermatozoa.

MATERIALS AND METHODS

SPERMATOZOA

The Department of Forensic Medicine, University of Lund, supplied the sperm samples which came from fertile men who were examined prior to sterilization. The ejaculates were produced by masturbation and delivered to our laboratory within 2 hours. They had been examined for sperm concentration, motility, and morphology. Only apparently normal semen specimens were used for our experiments. In order to purify sper-

Received July 13, 1983; revised and accepted February 28, 1984.

*Department of Obstetrics and Gynecology, University Hospital.

†Present address and reprint requests: Pål Wølner-Hanssen, M.D., Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, Washington 98195.

‡Department of Medical Microbiology, University of Lund.

matozoa, ejaculates were layered over 5 ml of a sucrose-phosphate buffer (2-SP)¹³ containing 7.5% Ficoll 400 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) followed by centrifugation at $500 \times g$ for 5 minutes and $1200 \times g$ for 10 minutes.¹⁴ The pellet was resuspended in 2-SP, and the concentration of spermatozoa was adjusted to 10^7 cells/ml.

MICROORGANISMS

Strains of *C. trachomatis*, immunotypes D, H, and I, that had been passaged in the yolk sac of embryonated hen's eggs, were tested. The strains were obtained from Dr. S.-P. Wang, University of Washington, Seattle, Washington. The infected yolk sacs were suspended in 2-SP, shaken with glass beads, and centrifuged at $1300 \times g$ for 15 minutes at 4°C. The intermediate phase was collected and centrifuged again under the same premises. The intermediate phase was collected and centrifuged at $20,000 \times g$ for 45 minutes. The pellet was resuspended in 2-SP and centrifuged at $1300 \times g$ for 15 minutes. The intermediate phase was collected and stored at -80°C until required. For adherence tests, chlamydial suspensions were thawed and centrifuged at $20,000 \times g$ for 55 minutes at 4°C. The pellet was resuspended to the original volume in 2-SP.

ADHERENCE TESTS

Unless otherwise indicated, the chlamydial suspension, containing $\sim 5 \times 10^6$ inclusion-forming units (IFU) of *C. trachomatis*/ml, was mixed with an equal amount of the spermatozoa suspension. The mixture was incubated for 45 minutes at 37°C on a shaker. Thereafter, it was layered over a 7.5% Ficoll 400 solution in 2-SP, and the spermatozoa were pelleted by centrifugation at $500 \times g$ for 5 minutes, then at $1200 \times g$ for 10 minutes. The pellet was resuspended in 1/15 M phosphate-buffered saline and washed twice by centrifugation.

In order to test the impact of pH shifts of the test environment on the adherence of *C. trachomatis* to the spermatozoa, the pH of the Ficoll solution, and of the two buffers used, namely 2-SP and phosphate-buffered saline, was varied by adding 1/15 M HCl or NaOH. In other tests, the number of chlamydiae in relation to spermatozoa was varied. The influence of the duration of incubation was also tested (as described below).

CONTROLS

To rule out the possibility that chlamydiae from an infected donor were already attached to the spermatozoa, a part of the semen was used as a control in each experiment. These spermatozoa were purified, incubated, and stained in the same way as the test specimens, except that no chlamydiae were added.

SPERM PENETRATION TESTS

Suspensions of *C. trachomatis* immunotype I, which contained $\sim 5 \times 10^6$ IFU/ml, were mixed with fresh, unprocessed semen and incubated for 45 minutes at 37°C on a shaker. Columns of albumen prepared from fresh hen's eggs were drawn into 10-cm-long capillary tubes (Vitrex, Swedish Hospital Supply, Gothenburg, Sweden), the inner diameter of which was 0.3 mm. One end of the tubes was sealed with modeling clay. The other end was immersed in a reservoir containing the incubated mixture of chlamydiae and semen. The tubes with the reservoirs were incubated in a moist chamber at 37°C for as long as 3 hours to allow a high number of spermatozoa to penetrate the albumen columns. Each tube was then cut into 2.5-cm-long pieces, and the part that had been immersed in the reservoir was discarded. The contents of each of the other pieces were spotted onto separate glass slides, allowed to dry, and fixed in acetone for 10 minutes. The specimens were stained with fluorescein-labeled antiserum, as described below.

IMMUNOFLUORESCENCE STAINING

One drop of the incubated and washed suspension of spermatozoa and chlamydiae was spotted onto glass slides, allowed to dry, and fixed in acetone for 10 minutes. The slides were then overlaid with fluorescein-labeled monoclonal antibodies to *C. trachomatis* (Mikro-Trak, *Chlamydia trachomatis* Culture Confirmation Test, Syva Company, Palo Alto, CA) and incubated for 30 minutes at 37°C in a moist chamber. The specimens were rinsed in 1/15 M phosphate-buffered saline for 10 minutes and in deionized water for 10 seconds. After staining, the glasses were allowed to dry and mounted with glycerin. A Leitz fluorescence microscope (Leitz AG, Wetzlar, West Germany) was used to study the specimens ($\times 1250$).

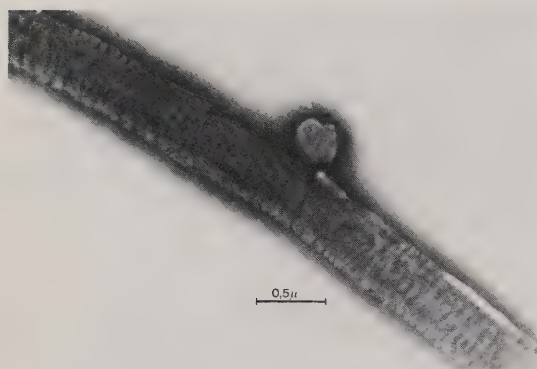


Figure 1
Micrograph of spermatid tail with attached chlamydial elementary body. Organisms negatively stained by phosphotungstic acid.

ELECTRON MICROSCOPY

For transmission electron microscopy, preparations of spermatozoa and chlamydiae similar to those described above were used. The samples were washed in distilled water containing 0.15% formaldehyde in order to remove sucrose constituents. The samples were stained with 2% aqueous phosphotungstic acid. One drop of the mixture was layered on copper grids coated with carbon platinum, which were left to dry after absorption of excess fluid with blotting paper. The preparations were examined in a Philips 300 electron microscope (Philips, Eindhoven, The Netherlands).

STATISTICAL ANALYSIS

Two-way analysis of variance was employed for statistical testing of the data.

RESULTS

As demonstrated by the immunofluorescence tests and electron-microscopic studies, chlamydiae adhere to spermatozoa in vitro (Figs. 1 and 2). The attached chlamydiae seemed to be randomly distributed over the spermatid body, without any preferential localization. We did not notice any morphologic differences between those spermatozoa that carried chlamydiae and those that did not.

Figure 3 shows that increasing acidity (i.e., from pH 4.0 to 8.0) significantly ($F = 15.7, 3 + 12$ df, $P < 0.001$) enhanced the proportion of sperm-



Figure 2
Micrograph of chlamydiae attached to spermatid tails and heads. Organisms stained by an immunofluorescence method ($\times 3000$).

tozoa that carried chlamydiae. This was also true ($F = 8.1, 3 + 12$ df, $P < 0.01$) for the number of chlamydiae attached to each spermatozoon that carried such organisms (Fig. 4).

In another test series, the effect on the adherence of variations in the concentration of *C. trachomatis* in the incubation medium was studied. When the number of chlamydiae in the medium was increased from 5×10^4 to 2×10^7 IFU/ml, the proportion of spermatozoa that carried such organisms increased significantly ($F = 19.6, 3 + 9$ df, $P < 0.001$) (Fig. 5). Even the number of chla-

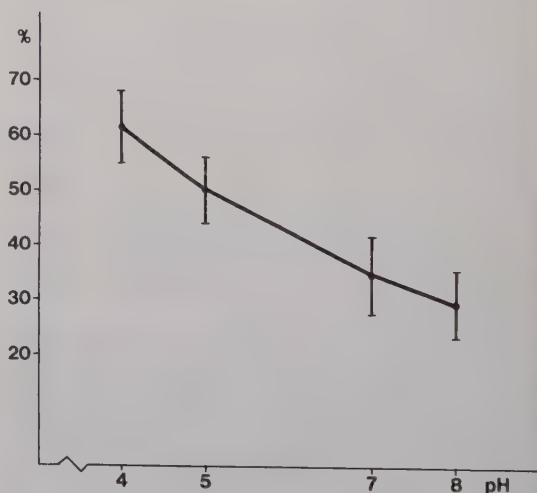


Figure 3
Percentage of spermatozoa carrying chlamydiae after incubation at various pH levels of the test medium.

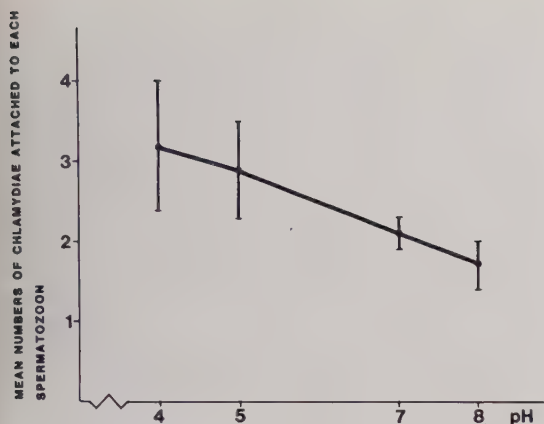


Figure 4
Mean numbers of chlamydiae attached to individual spermatozoa after incubation at various pH levels of the test medium.

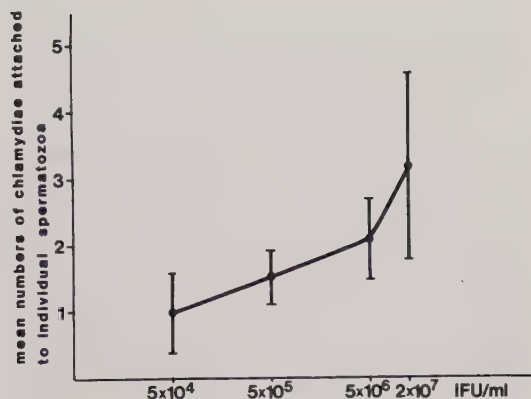


Figure 6
Mean number of chlamydiae attached to individual spermatozoa after incubation with various concentrations of chlamydial cells.

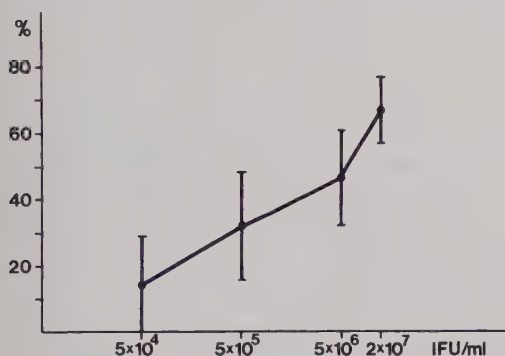


Figure 5
Percentage of spermatozoa carrying chlamydiae after incubation with various concentrations of chlamydial cells.

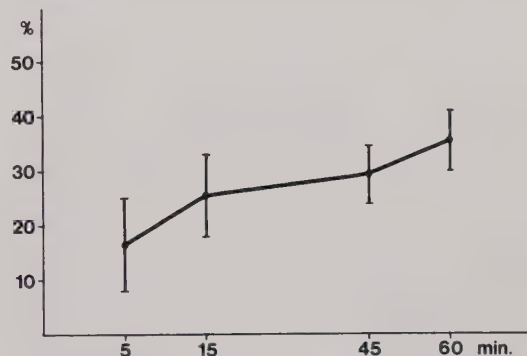


Figure 7
Percentage of spermatozoa carrying chlamydiae after various incubation times.

mydiae adhering to individual spermatozoa correlated ($F = 5.8$, $3 + 9$ df, $P < 0.05$) with the concentration of chlamydiae used in the adherence tests (Fig. 6).

When we used a test medium kept at pH 7.0, to which a concentration of 5×10^6 IFU of chlamydiae and 10^7 spermatozoa/ml had been added, increasing incubation times (e.g., 5, 15, 45, and 60 minutes) gave significantly ($F = 8.6$, $3 + 9$ df, $P < 0.01$) increasing percentages of spermatozoa that carried chlamydiae (Fig. 7).

Immunofluorescence staining of the control specimens usually revealed no evidence of attached chlamydiae. However, in two tests, 6% and 7% of the spermatozoa, respectively, seemed to carry chlamydiae attached to them.

Examination of the albumen columns from the capillary tubes showed that an average of 25% of the spermatozoa that had progressed 25 to 75 mm carried chlamydiae attached to them.

DISCUSSION

This study indicates that chlamydiae can adhere to spermatozoa under experimental conditions, as evidenced by immunofluorescence tests and transmission electron microscopic studies.

In studies of the attachment of various bacteria to human urothelial cells, the number of bacteria adhering to each cell was found to increase in proportion to the bacterial density in the test en-

vironment within the range of 10^5 to 10^{11} bacteria/ml.¹⁵ Our experiments showed a similar phenomenon. Thus, an increasing concentration of chlamydiae in the incubation medium enhanced the number of spermatozoa carrying such organisms. This also accounts for the number of chlamydiae that attached to individual spermatozoa. The mean number of chlamydiae that attached to an individual spermatozoon carrying such organisms rarely exceeded four (Figs. 4 and 6). This might be explained by the relatively few chlamydiae used in the test system, compared with the number of spermatozoa (maximum 2×10^7 IFU of chlamydiae versus 10^7 spermatozoa/ml).

This study showed that by lengthening the incubation time (from 5 to 60 minutes), the number of chlamydiae that attached to spermatozoa increased significantly. In a previous study,¹⁶ the association of *C. trachomatis* with McCoy cell monolayers increased linearly for 60 minutes and then stopped almost completely. That *Chlamydia psittaci* attached to the surface of L cells decreased in number when the incubation time exceeded 30 minutes was explained by the more rapid ingestion of already attached chlamydiae than the rate of further attachment.¹⁷ There is no evidence that spermatozoa can ingest chlamydiae. Thus, such cells might be useful for further studies on chlamydial attachment.

Attachment of bacteria to eukaryotic cells is dependent on close-range contact ($< 4 \text{ \AA}$).¹⁸ Such contact is counteracted by repulsive forces due to, among other things, the surface charge of the cells and bacteria. Increasing acidity of the surrounding environment induces reduction of the cell surface charge and, accordingly, might weaken the repulsive forces, with a consequent increased chance of microbial attachment.¹⁸ In the present study, we investigated the influence of pH shifts on the attachment of chlamydiae to spermatozoa. With decreasing pH (from 8.0 to 4.0), the proportion of spermatozoa carrying chlamydiae, as well as the mean number of chlamydiae attached to each spermatozoon, increased significantly. These observations are in accordance with the finding of an isoelectric point at pH 4.64 for elementary bodies of *C. trachomatis*, immunotype D.¹⁹ The isoelectric points for ejaculated ram and bull spermatozoa have been estimated to be at pH 4.9²⁰ and 4.7,²¹ respectively. (To the best of our knowledge, the isoelectric point for human spermatozoa has not been reported.)

If chlamydiae can attach to spermatozoa in vivo also, the place for a "rendezvous" between the two cell types might, in the male, be in the urethra and the accessory genital glands, and in the female, in the vagina and the uterine cervix. The alkaline ejaculate is probably a comparatively unsuitable environment for chlamydial adherence. The posterior vaginal vault, which is usually an acidic environment, seems a more likely site for such adherence. However, the vaginal contents of women with genital infection, which—at least in a proportion of such patients—have an abnormally high pH, might offer a slightly reduced chance for chlamydiae to adhere to spermatozoa, compared with such contents of noninfected women.

It has been hypothesized that gonococci can be transported from the seminal vesicles to the epididymis by "hitchhiking" on spermatozoa and thus cause epididymitis.¹² In an in vitro model, passage of spermatozoa through cervical secretion facilitated transport of bacteria through the mucus.²² In that study, it was not established, however, whether the bacteria also adhered to the spermatozoa or just moved along with them through the cervical mucus. In the present study, it was demonstrated that spermatozoa, when progressing forward through an albumen column, could carry chlamydiae attached to them.

Whether chlamydiae can also attach to spermatozoa in vivo and whether organisms attached in this way can be transported through the genital tract remains to be studied. Even if the microorganisms do "hitchhike" in vivo, salpingitis might not result unless a certain minimum required dose of chlamydiae reaches the upper genital tract. This dose is not yet known.

Acknowledgments. We thank Eva Sandgren and Christina Pehrson for excellent technical assistance. We also thank Jarl Ekstrand for valuable advice and skilled assistance with regard to electron microscopy.

REFERENCES

1. Mårdh P-A, Möller BR, Paavonen J: Chlamydial infection of the female genital tract with emphasis on pelvic inflammatory disease: a review of Scandinavian studies. *Sex Transm Dis* 8:140, 1981
2. Oriel JD: Infections of the male genital tract. In *Chlamydial Infections*, Edited by P-A Mårdh, KK Holmes, JD Oriel, P Piot, J Schachter. Amsterdam, Elsevier Biomedical Press, 1982, p 93

3. Mårdh P-A, Møller BR, Ingerslev HJ, Nüssler E, Weström L, Wølner-Hanssen P: Endometritis caused by *Chlamydia trachomatis*. Br J Vener Dis 57:191, 1981
4. Wølner-Hanssen P, Mårdh P-A, Møller BR, Weström L: Endometrial infection in women with chlamydial salpingitis. Sex Transm Dis 9:84, 1982
5. Mårdh P-A, Ripa KT, Svensson L, Weström L: *Chlamydia trachomatis* infection in patients with acute salpingitis. N Engl J Med 296:1377, 1977
6. Berger RE, Alexander ER, Monda GD, Ansell J, McCormick G, Holmes KK: *Chlamydia trachomatis* as a cause of acute "idiopathic" epididymitis. N Engl J Med 298:301, 1978
7. Møller BR, Mårdh P-A: Experimental epididymitis and urethritis in grivet monkeys provoked by *Chlamydia trachomatis*. Fertil Steril 34:275, 1980
8. James-Holmquest AN, Swanson J, Buchanan TM, Wende RD, Williams RP: Differential attachment by piliated and nonpiliated *Neisseria gonorrhoeae* to human sperm. Infect Immun 9:897, 1974
9. James AN, Knox JM, Williams RP: Attachment of gonococci to sperm: influence of physical and chemical factors. Br J Vener Dis 52:128, 1976
10. Gomez CI, Stenback WA, James AN, Criswell BS, Williams RP: Attachment of *Neisseria gonorrhoeae* to human sperm: microscopical study of effect of trypsin and iron. Br J Vener Dis 55:245, 1979
11. Gnärpe H, Friberg J: T-mycoplasmas on spermatozoa and infertility. Nature 245:97, 1973
12. Howard TL: Bacterial hitch-hikers. J Urol 106:94, 1971
13. Gordon FB, Harper IA, Quan AL, Treharne JD, Dwyer RStC, Garland JA: Detection of *Chlamydia (Bedsonia)* in certain infections of man. I. Laboratory procedures: comparison of yolk sac and cell culture for detection and isolation. J Infect Dis 120:451, 1969
14. Harrison RAP: A highly efficient method for washing mammalian spermatozoa. J Reprod Fertil 48:347, 1976
15. Mårdh P-A, Colleen S, Hovelius B: Attachment of bacteria to exfoliated cells from the urogenital tract. Invest Urol 16:322, 1979
16. Lee CK: Interaction between a trachoma strain of *Chlamydia trachomatis* and mouse fibroblasts (McCoy cells) in the absence of centrifugation. Infect Immun 31:584, 1981
17. Byrne GI: Kinetics of phagocytosis of *Chlamydia psittaci* by mouse fibroblasts (L cells): separation of the attachment and ingestion stages. Infect Immun 19:607, 1978
18. Colleen S, Mårdh P-A: Bacterial colonization of human urethral mucosa. II. Adherence tests using tissue organ cultures. Scand J Urol Nephrol 15:181, 1981
19. Kraaiipoel RJ, van Duin AN: Isoelectric focusing of *Chlamydia trachomatis*. Infect Immun 26:775, 1979
20. Hammerstedt RH, Keith AD, Hay S, DeLuca N, Amann RP: Changes in ram sperm membranes during epididymal transit. Arch Biochem Biophys 196:7, 1979
21. Hammerstedt RH, Keith AD, Boltz RC Jr, Todd PW: Use of amphiphilic spin labels and whole cell isoelectric focusing to assay charge characteristics of sperm surfaces. Arch Biochem Biophys 194:565, 1979
22. Toth A, O'Leary WM, Ledger W: Evidence for microbial transfer by spermatozoa. Obstet Gynecol 59:556, 1982

INFLUENCE OF AMNIOTIC FLUID ON THE FORMATION OF CHLAMYDIAL INCLUSIONS IN MCCOY CELL CULTURES

PÅL WØLNER-HANSEN¹, LARS WESTRÖM¹, AND PER-ANDERS MÅRDH²

¹Department of Obstetrics and Gynecology, University Hospital, Lund and

²Department of Medical Microbiology, University of Lund, Lund (Sweden)

INTRODUCTION

Colonization with Chlamydia trachomatis has been reported in up to one fourth of puerperal women (1). Passing through an infected genital tract during parturition, the fetus can become contaminated with chlamydiae, and might later develop inclusion conjunctivitis (2) and/or pneumonia (3).

Puerperal salpingitis has been reported in mothers whose infants had inclusion conjunctivitis (2).

Body fluids, e.g. amniotic fluid (4,5) and seminal plasma (6,7), show antibacterial activity in vitro. Seminal plasma and some of its constituents, such as spermine and zinc ions, might also interfere with the capacity of C. trachomatis to form inclusions in McCoy cells (8).

The purpose of the present study was to investigate whether amniotic fluid, collected during different gestation periods, could inhibit the formation of chlamydial inclusions in McCoy cell cultures, and if so, to characterize factors responsible for the inhibition.

MATERIALS AND METHODS

Amniotic fluid. The specimens were collected by abdominal amniocentesis or when performing Caesarian section. Antibiotics and chemotherapeutics had not been administered to any of the patients studied. The amniotic fluid was stored at -20°C until required.

Organism. A strain of C. trachomatis, immunotype E, that had been passed in the yolk sac of embryonated hen's eggs was tested. The strain was originally obtained from Dr. S.-P. Wang, Seattle, U.S.A.

Culture Technique. The chlamydiae were inoculated onto McCoy cells (originally obtained from the Institute of Ophthalmology, University of London, UK). Complete Medium Glucose Antibiotics (CMGA) was used as culture medium. The CMGA contained RPMI 1640 (Flow Laboratories Ltd., Scotland) with inactivated calf serum, 5 g glucose, 25 mg fungizone, 100 mg gentamicin, and 29 g glutamine per litre medium. The cells were cycloheximide-treated as previously described (9). The inoculated cell cultures were centrifugated at 4,000 rpm for 60 min-

utes, and incubated at 37°C for 72 hours. The cells were studied under a light-microscope after being fixed with methanol and stained with iodine.

The above-mentioned culture method was also modified as follows. Pooled amniotic fluid from the 16th-17th, and 39th-41st week of pregnancy was added to CMGA in dilutions of 10%, 20%, 40%, 60%, and 80%. Such mixture or pure amniotic fluid was used as tissue culture medium. To exclude the possibility of inhibition of chlamydial growth due to nutrient deficiency by dilution of the tissue culture medium, pooled amniotic fluid was freeze-dried. The lyophilized preparation was restored to the original volume by adding CMGA. The mixture was used as such, or else it was diluted with CMGA to prepare a suspension that was equivalent to concentrations of 10%, 20%, 40%, 60%, and 80% of amniotic fluid in tissue culture medium. In order to determine whether amniotic fluid acts on chlamydial elementary bodies (EB), chlamydiae were incubated with dilutions of pooled amniotic fluid for approximately 18 hours at 37°C. The mixture was then centrifuged at 15,000 rpm for 60 minutes. This deposit was inoculated onto McCoy cell cultures. To test whether the inhibitory factors in amniotic fluid were heat stable, pooled fluid was heated at 80°C for 20 minutes before it was diluted and added to the cell cultures. Finally, we dialysed pooled amniotic fluid in a tube with a pore size of 25 Å, using distilled water as external fluid. The dialysate was added to CMGA in the concentrations given above.

RESULTS

Added to the tissue culture medium amniotic fluid from women in the second and the third trimester was found to inhibit the formation of chlamyial inclusions (Figures 1a and 1b). The inhibitory activity of amniotic fluid was similar in both the gestational periods tested. The McCoy cells did not show any cytopathogenic effect (CPE) when exposed to 100% amniotic fluid.

The experiments with freeze-dried amniotic fluid indicate that the inhibitory activity of amniotic fluid was not attributable to dilution of the tissue culture medium (Figure 1c). Preincubation of the test organisms in amniotic fluid for approximately 18 hours had a negative effect on the formation of chlamydial inclusions (Figure 1d). The chlamydia-inhibitory principal in amniotic fluid resisted heating for 20 minutes at 80°C (Figure 1e). Dialysis of amniotic fluid against distilled water revealed that the antichlamydial principal did not pass 25Å pores (Figure 1f).

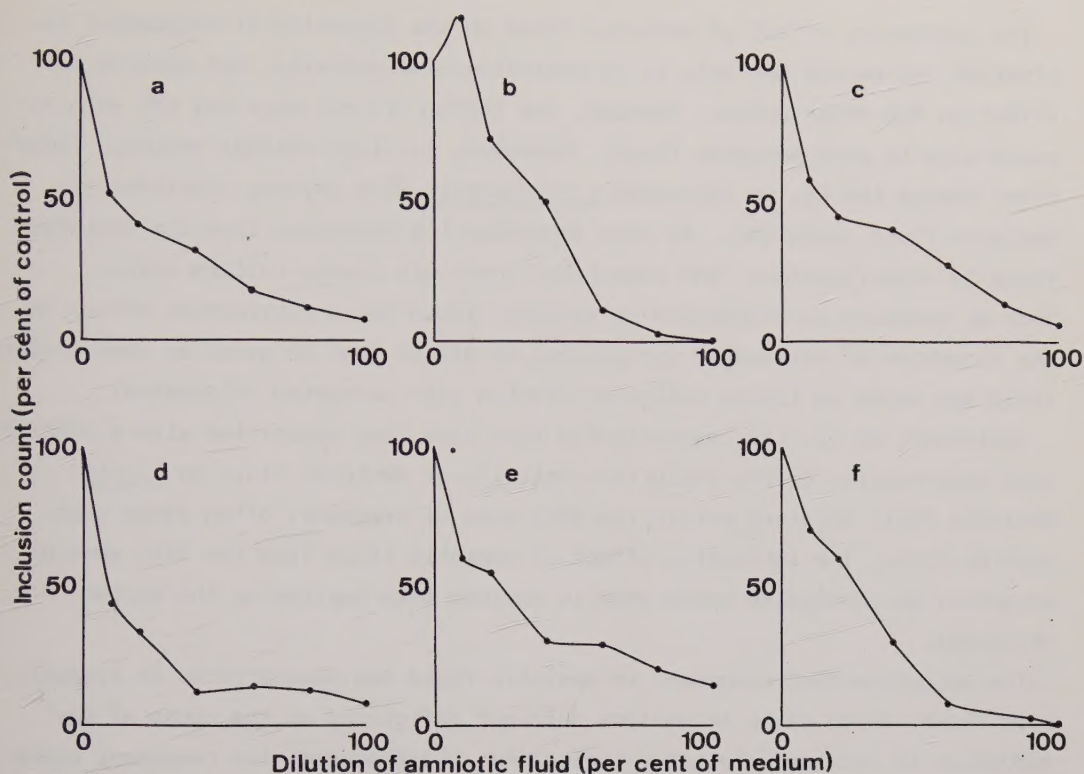


Fig. 1. Inclusion count (in per cent of inclusion count in control cultures) of *C. trachomatis*, immunotype E, in McCoy cell cultures to which amniotic fluid from the 16-17th (a) or 39-41th (b) week of pregnancy was added to the tissue culture medium in increasing concentrations. Second trimester amniotic fluid was also either freeze-dried (c), heated at 80°C for 20 minutes (d), or dialysed (e) before adding to the medium. Incubation of chlamydiae with dilutions of amniotic fluid overnight before they were inoculated onto the cell cultures was also performed (f).

COMMENTS

The present investigation demonstrated that amniotic fluid can inhibit the formation of intracytoplasmatic inclusions in McCoy cell cultures. The results are in principle in agreement with other studies that have shown antibacterial activity of amniotic fluid on facultatively anaerobic (e.g. *Escherichia coli*) and strictly anaerobic (e.g. *Bacteroides fragilis*) bacteria (4,5). Our results indicate that the inhibitory activity of amniotic fluid on chlamydiae is present as early as the 16th week of pregnancy. This is contrary to the effect of such fluid on *E. coli* (4). Amniotic fluid obtained before the 26th week of gestation proved non-inhibitory for this latter organism; whereas samples obtained before the 20th week stimulate bacterial growth (4). Thadepalli et al. (5) also demonstrated a growth-stimulating effect of second trimester (16-20 weeks of gestation) amniotic fluid on *E. coli*.

The inhibitory effect of amniotic fluid on the formation of chlamydial inclusions can be due not only to an antichlamydial activity, but also to an effect on the McCoy cells. However, the latter did not show any CPE when exposed even to pure amniotic fluid. Moreover, to study whether amniotic fluid might damage the EB, we incubated C.trachomatis with various dilutions of amniotic fluid overnight. We then separated the organisms from the amniotic fluid by centrifugation and inoculated them onto tissue culture cells. Such an exposure of chlamydiae to amniotic fluid had a detrimental effect on the formation of chlamydial inclusions; an effect just as great as when amniotic fluid was added to tissue cultures infected with untreated chlamydiae.

Schlievert et al. (10) demonstrated that zinc ions associated with a peptide were responsible for the inhibitory activity of amniotic fluid on E.coli. Amniotic fluid obtained before the 20th week of pregnancy often lacks this peptide (10). The inhibiting effect of amniotic fluid from the 16th week of pregnancy on chlamydiae would seem to exclude this peptide as the active principal.

The antichlamydial component in amniotic fluid was demonstrated in lyophilized fluid, which seems to exclude nutrient deficiency as the cause of the reduction in intracytoplasmatic inclusions. Furthermore, the component seems to be comparatively heat-stable and of a relatively large molecular size - not easily passing 25 Å pores.

REFERENCES

1. Mårdh, P.-A., Helin, I., Bobeck, S., Laurin, J., Nilsson, T. (1980) Br. J. Vener. Dis., 56, 96.
2. Rees, E., Tait, I.A., Hobson, D., Johnson, F.W.A. (1977) in: Hobson, D., Holmes, K.K. (Eds.), Nongonococcal Urethritis and Related Infections, American Society for Microbiology, Washington, D.C., pp. 140-147.
3. Beem, M.O., Saxon, E.M. (1977) N. Engl. J. Med., 296, 306.
4. Larsen, B., Snyder, I.S., Galask, R.P. (1974) Am. J. Obstet. Gynecol., 119, 492.
5. Thadepalli, H., Bach, V.T., Davidson, E.C. (1978) Obstet. Gynecol., 52, 198.
6. Mårdh, P.-A., Colleen, S. (1975) Scand. J. Urol. Nephrol., 9, 17.
7. Stamey, T.A., Fair, W.R., Timothy, M.M., Chung, H.K. (1968) Nature, 218, 444.
8. Mårdh, P.-A., Colleen, S., Sylwan, J. (1980) Invest. Urol., 17, 510.
9. Ripa, K.T., Mårdh, P.-A. (1977) J. Clin. Microbiol., 6, 238.
10. Schlievert, P., Johnson, W., Galask, R.P. (1977) Am. J. Obstet. Gynecol., 127, 603.

